EFFECT OF HYDROXYL RADICAL SCAVENGING ON ENDOTOXIN-INDUCED LUNG INJURY

The release of oxygen radicals, in particular the hydroxyl radical, from sequestered neutrophils produces acute lung injury after a number of insults. Our purpose was to determine whether hydroxyl radical $OH^{\cdot}$ is responsible for the lung injury from endotoxin characterized by (1) pulmonary leukostasis, (2) increased thromboxane production leading to pulmonary hypertension and hypoxia, and (3) increased protein permeability. This hypothesis was tested by infusion of a selective $OH^{\cdot}$ scavenger, dimethyl thiourea (0.75 gm/kg), into unanesthetized sheep before endotoxin and comparing the response to that seen with endotoxin alone. Pulmonary vascular integrity was measured by the use of lung lymph flow, Q_L, lymph protein transport, Thromboxane A_2 was measured as TxB_2, and prostacyclin as 6-keto-$PGF_{1\alpha}$. We found no difference in the degree of leukopenia and hypoxia after endotoxin or the levels of TxB_2, 6-keto-$PGF_{1\alpha}$ and pulmonary hypertension with dimethyl thiourea, compared with endotoxin alone. The permeability injury was also identical, with a two-fold to three-fold increase in protein-rich lymph seen in both groups. It appears that $OH^{\cdot}$ does not play a major causative role in either phase of endotoxin lung injury.

Wong, C., *Fox, R.*, and Demling, R. H.

Surgery 97(3):300-306, 1985.

Other support: National Institutes of Health.

From the Longwood Area Trauma Center, Beth Israel Hospital, Harvard Medical School, and Brigham and Women's Hospitals, and the Children's Hospital, Boston.

PROSTAGLANDINS AND INTRACELLULAR CYCLIC AMP IN RESPIRATORY SECRETORY CELLS

Prostaglandins are known to affect ion transport and mucus secretion in the trachea, and at least part of their effect is thought to be mediated by cyclic AMP. Because no prostaglandin receptor assay is currently available, we have used an immunocytochemical probe for the intracellular localization of cyclic AMP to identify those specific cell types in dog and cat trachea that respond to prostaglandins. Using tracheal explants similar to those used by other investigators for *in vitro* studies of ion transport and glycoprotein secretion, we examined the effect of endogenous and exogenous prostaglandins on immunoreactive cyclic AMP. Endogenous prostaglandins, which may be secreted in response to minor distortion of tissue membranes, stimulated immunoreactive cyclic AMP in ciliated epithelial cells and in both serous and mucous submucosal gland cells. This was not observed in tissues assayed 60 min after dissection or dissected in the presence of indomethacin or of BW755C. Exogenous prostaglandins increased cyclic AMP in these same cell types. We conclude that prostaglandins stimulate cyclic AMP in specific cell types in the trachea, and that endogenous prostaglandins play a major role in many *in vitro* preparations.

Lazarus, S. C. Basbaum, C. B. and *Gold, W. M.*

American Review of Respiratory Disease 130:262-266, 1984.

Other support: National Heart, Lung and Blood Institute.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

From the Cardiovascular Research Institute and the Departments of Medicine and Anatomy, University of California, San Francisco.

AIRWAY RESPONSIVENESS TO INHALED ANTIGEN, HISTAMINE, AND METHACHOLINE IN INBRED, RAGWEED-SENSITIZED DOGS

The authors studied the responses to antigen in animals selected from a colony of inbred dogs sensitized to specific allergens to determine if they had characteristics similar to those of human asthmatics. They were immunized with ragweed and grass pollen extracts (10 μg in alum) immediately after routine vaccination with attenuated live virus (distemper and hepatitis) and killed bacteria (Leptospira) at 4, 8, and 12 wk of age. Subsequently, ragweed and grass injections were repeated every 2 months. Immunized dogs made specific IgE-antibodies in serum averaging 3 to 4 times that of control animals (no immunization with pollen or vaccine). They showed positive skin responses to the injection of ragweed pollen extract, whereas control dogs did not respond to ragweed pollen by quantitative skin test or inhalation challenge. In immunized dogs under barbiturate anesthesia, air-flow resistance of the total respiratory system increased from 0.60 ± 0.07 (mean ± SEM) before to 12.6 ± 3.4 cm H_2O/lps 5 min after the start of antigen aerosol; respiratory resistance remained increased for 20 min and was associated with hypoxemia and increased arterial plasma histamine. In addition, airway responsiveness to both inhaled histamine and methacholine was greater in immunized dogs than in nonimmunized dogs of comparable age. Airway responses to each agonist were highly reproducible on repeated testing. These results indicate that physiologic responses to antigen by inbred, ragweed-sensitized dogs resemble human asthma closely and that these dogs appear suitable for a variety of experimental studies of asthma with respect to pathogenesis, diagnosis, prevention, and treatment.

Mapp, C., Hartiala, J., Frick, O. L. Shields, R. L., and *Gold, W. M.*

American Review of Respiratory Disease 132:292-298, 1985.

Other support: National Lung and Blood Institute, National Institute of Allergy and Infectious Diseases, and the Northern California Allergy Society.

From the Cardiovascular Research Institute and Departments of Medicine and Pediatrics, University of California, San Francisco, and the Animal Resources Services Facility of the School of Veterinary Medicine, University of California, Davis.

CHARACTERIZATION OF PURIFIED DOG MASTOCYTOMA CELLS: AUTONOMIC MEMBRANE RECEPTORS AND PHARMACOLOGIC MODULATION OF HISTAMINE RELEASE

There is conflicting evidence as to which autonomic receptors mast cells possess and whether the receptors are capable of modulating mediator release. The authors have studied dog mastocytoma cells because they are available in large numbers in a relatively pure form, unlike normal dog mast cells. Mastocytoma nodules from a dog were excised and disaggregated with collagenase to provide a cell suspension of mastocytoma cells of ≥ 92% purity. The presence of autonomic receptors was assessed by both radioligand binding assays and by evaluating pharmacologic modulation of mediator release. In the radioligand binding assays, beta-adrenergic receptors were estimated by [^{3}H]prazosin binding and cholinergic receptors by [^{3}H]quinuclidinyl benzilate binding. Nonspecific binding was determined in each case by incubation in the presence of the specific

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

antagonists propranolol, phentolamine, and atropine, respectively. The effect of autonomic agonists on immunologic and nonimmunologic histamine release was examined using the beta-adrenergic agonists isoproterenol and terbutaline, the alpha-adrenergic agonist phenylephrine with and without propranolol, and the cholinergic agonist acetylcholine. Dose-response curves were constructed both for the autonomic agonists and the histamine-releasing agents. Results from the radioligand binding and the pharmacologic studies were concordant. These dog mastocytoma cells had a high density of beta-receptors (21,500 ± 3,300; mean ± SE beta-receptors/cell, n=5) of which the predominant subtype appeared to be $beta_2$. No evidence was found for the presence of alpha-adrenergic or cholinergic receptors either by direct receptor binding or by their actions on histamine release.

Phillips, M. J., Barnes, P. J., and *Gold, W. M.*

American Review of Respiratory Disease 132:1019-1026, 1985.

Other support: National Heart, Lung and Blood Institute, the Strobel Medical Research Fund of the American Lung Association of San Francisco, the University of California Research Evaluation and Allocation Committee, and the California Research and Medical Education Fund of the American Lung Association of California.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

NICOTINE-INDUCED RESPIRATORY EFFECTS OF CIGARETTE SMOKE IN DOGS

The authors report that nicotine is responsible for both a blood-borne stimulation of the respiratory center and a direct effect on intrathoracic airway tone in dogs. They introduced cigarette smoke into the lungs of donor dogs and injected arterial blood obtained from them into the circulation of recipient dogs to show that a blood-borne material increased breathing and airway smooth muscle tone. Smoke from cigarettes containing 2.64 mg of nicotine was effective; that from cigarettes containing 0.42 mg of nicotine was not. Nicotine, in doses comparable to the amounts absorbed from smoke, also increased breathing and tracheal smooth muscle tension when injected into the vertebral circulation of recipient dogs. Finally, blockade of nicotine receptors in the central nervous system and in the airway parasympathetic ganglia inhibited the effects of inhaled cigarette smoke and intravenous nicotine on the respiratory center and on bronchomotor tones. The authors concluded that nicotine absorbed from cigarette smoke is the main cause of cigarette smoke-induced bronchoconstriction. It caused central respiratory stimulation, resulting in increased breathing and airway smooth muscle tension, and had a direct effect on airway parasympathetic ganglia as well.

Hartiala, J. J., Mapp, C., Mitchell, R. A., and *Gold, W. M.*

Journal of Applied Physiology 59(1):64-71, 1985.

Other support: National Heart, Lung, and Blood Institute.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

AN ULTRASTRUCTURAL ANALYSIS OF DOG MASTOCYTOMA CELLS AND NORMAL MAST CELLS

A well-differentiated dog mastocytoma was characterized ultrastructurally using morphometric, histochemical, and biochemical methods. The ultrastructure of cells in the intact tumor was compared to the morphology of cells disaggregated from the tumor and cultured for periods as long as 4 weeks and to normal dog connective tissue mast cells. Most of the tumor cells contained histamine (mean = 5.81 pg/cell), demonstrated chloroacetate esterase activity histochemically, stained metachromatically with toluidine blue, and were similar in ultrastructure to normal dog mast cells. The proportion of mast cells in this tumor averaged 67%; eosinophils, fibroblasts, plasma cells, and macrophages also were present. The mean diameter of mast cells (12.79 μm) and the mean diameter of their cytoplasmic granules (473 nm) were similar to those reported for mast cells and mastocytoma cells from various species. The heterogeneity in appearance of the mastocytoma granules was consistent with a variable degree of granule maturation. After disaggregation or periods of culture ranging from 2 days to 4 weeks, the mean granule diameters were 15% larger than those measured in the intact mastocytoma cells, though other morphologic features remained unchanged. Although the cells retained their distinct morphological features for at least 4 weeks, some of their physiological responses were lost after 1 week in culture. This study showed that dog mastocytomas can be a source of a large, relatively homogeneous population of cells that are useful for elucidating some of the structural and functional properties of mast cells.

Calonico, L. D., Phillips, M. J., McDonald, D. M., and *Gold, W. M.*

The Anatomic Record 212:399-407, 1985.

Other support: National Heart, Lung and Blood Institute, the Strobel Medical Research Fund of the American Lung Association of San Francisco, and the University of California Research and Medical Education Fund of the American Lung Association of California.

From the Cardiovascular Institute and Departments of Anatomy and Medicine, University of California, San Francisco.

IMMUNOLOGIC CHALLENGE AND EPITHELIAL ION TRANSPORT IN CANINE TRACHEA

In this study the authors used measured changes in short-circuit current to reflect ion transport across the canine tracheal epithelium and have shown that mediators released from lung fragments by immunologic challenge stimulate ion transport in the trachea. The presence of histamine in the supernatants, although the concentration is too low to account for the short-circuit current effect, indicates that mast cell degranulation has occurred. Particularly, the observation that the increase in short-circuit current can be blocked by pretreatment of the epithelial tissue with the cyclooxygenase inhibitor indomethacin suggests that the mediator effect on short-circuit current is *indirect* and is produced by activating the cyclooxygenase pathway in the epithelium.

Lazarus, S. C., Leikauf, G. D., McCabe, L. J., Paige, K., Chung, K. F., Nadel, J. A., and *Gold, W. M.*

Chest 287S:188S-189S, 1985.

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Other support: National Heart, Lung and Blood Institute, the California Research and Medical Education Fund of the American Lung Association of California, and the Research Evaluation and Allocation Committee of the University of California School of Medicine, San Francisco.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

PROTECTIVE REFLEXES AND VASCULAR EFFECTS IN THE NASAL MUCOSA ELICITED BY ACTIVATION OF CAPSAICIN-SENSITIVE SUBSTANCE P-IMMUNOREACTIVE TRIGEMINAL NEURONS.

The aim of the present investigation was to study the structure and functional characteristics of the sensory pathways which provide protective reflexes in the nasal mucosa in various experimental animals. The use of capsaicin desensitization as a possible treatment against nasal irritation has also been evaluated. The following specific aspects were studied.

(1) The origin and distribution of SP-IR nerve fibres in the nasal mucosa were investigated in several species, including man. Also the effect of systemic capsaicin pretreatment on SP-containing neurons was studied.

(2) The effect of antidromic trigeminal and parasympathetic nerve stimulation on cat nasal blood flow was compared with the response seen following local infusions of SP, VIP and capsaicin.

(3) The effects of antidromic trigeminal nerve stimulation, local application of SP or capsaicin on plasma extravasation in the nasal mucosa were studied in control and capsaicin-pretreated rats.

(4) The effect of cigarette smoke and its various components on nasal mucosal permeability was studied in the rat. Additionally, the sensory mechanisms underlying the avoidance behavior (nose-wipings) induced by cigarette smoke was investigated in the guinea-pig.

(5) The mechanisms underlying sneezing responses to both chemical irritants and tactile stimulation were studied following capsaicin pretreatment.

(6) The acute and long-term effects of local systemic capsaicin pretreatment and cryosurgery on the nasal mucosa were studied. The experiments examined the inhibition of nasal irritation, loss of SP-IR neurons, neurogenic plasma extravasation, local tissue damage and systemic effects following these treatments.

(7) The importance of capsaicin-sensitive sensory nerves for the cardiovascular responses induced by local application of nicotine and capsaicin to the nasal mucosa was also investigated.

Lundblad, L. *(Lundberg, J. M.)*

Acta Physiologica Scandinavica Supplement 529:1-42, 1984.

Other support: The Swedish Medical Research Council, the Swedish Tobacco Company, the Astra Foundation and the Karolinska Institute.

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From the Department of Pharmacology, Karolinska Institute, and the Department of Oto-rhino-laryngology, Karolinska Hospital, Stockholm, Sweden.

EVIDENCE FOR SUBSTANCE P-IMMUNOREACTIVE SPINAL AFFERENTS THAT MEDIATE BRONCHOCONSTRICTION

The origin and functional role of capsaicin-sensitive substance P-(SP-) immunoreactive (IR) nerve fibres in the lower airways were studied in the guinea-pig. Stellatectomy caused a significant reduction of SP-IR in the lung and pulmonary artery. Immunohistochemical analysis, however, did not reveal any clear-cut change in the number and distribution of SP-IR fibres in the lung of these animals. After combined stellatectomy plus local capsaicin treatment of the vagal nerves, most SP-IR nerves disappeared in the lower airways. The bronchoconstriction induced by capsaicin was significantly reduced after stellatectomy and abolished after stellatectomy plus capsaicin pretreatment of the vagal nerves. Ether inhalation caused bronchoconstriction, which was not influenced by stellatectomy but markedly reduced by combined capsaicin treatment of vagal nerves and stellatectomy. Stellate ganglion stimulation in animals that had been chemically sympathetctomized by 6-OH-dopamine caused bronchoconstriction, which was resistant to cholinergic or adrenergic receptor blockade. This response was absent after systemic capsaicin pretreatment, suggesting that it was due to antidromic stimulation of afferent fibres traversing the stellate ganglion. In conclusion, the present data suggest that the lower airways receive SP-IR capsaicin-sensitive C-fibre afferents of both vagal and spinal origin. These sensory fibres seem to have branches both within the bronchial smooth muscle and around blood vessels.

Saria, A., Martling, C.-R., Dalsgaard, C.-J., and *Lundberg, J. M.*

Acta Physiologica Scandinavica 125:407-414, 1985.

Other support: The Swedish Tobacco Company, the Gustaf V. Foundation, the Astra Foundation, the Petrus and Augusta Hedlunds Fonds, the Karolinska Institute, Austrian Scientific Research Funds, and the Swedish Medical Research Council.

From the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria; the Departments of Pharmacology and Anatomy, Karolinska Institute; and the Department of Anesthesiology, Karolinska Hospital, Stockholm, Sweden.

SEQUENCE OF PATHOLOGIC CHANGES IN THE AIRWAY MUCOSA OF GUINEA PIGS DURING OZONE-INDUCED BRONCHIAL HYPERREACTIVITY

The authors assessed the nature and progression of airway mucosal disease and muscarinic bronchial reactivity in guinea pigs studied in groups of 4 at 2h, 6h, 14h, 1 day, 2 days, or 4 days after ozone exposure (3.0 ppm for 2h), and in 1 control group. Muscarinic reactivity was determined by measuring specific airway resistance as a function of increasing doses of intravenous acetylcholine in 31 intact, unanesthetized, spontaneously breathing animals. After testing, each group was killed to obtain tracheal tissue for light microscopic examination. We found that airway hyperreactivity to acetylcholine occurred in 96% of the animals exposed to ozone. Its degree at 2h was substantial. Complete remission was not observed until the fourth day. In association with the acute bronchial hyperreactivity found at 2h, a marked decrease in airway mucosal goblet cells and an increase in mucosal mast cells occurred. Neutrophilic infiltration occurred later and lasted longer, despite remission of the hyperreactivity.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

Our results indicate that acute, ozone-induced bronchial hyperreactivity is related to signs of airway mucosal injury and mast cell infiltration. After this early phase of airway damage, neutrophilc infiltration occurs and persists, suggesting that it is a consequence of the damage rather than a cause of the increased airway reactivity after ozone exposure.

Murlas, C. and Roum, J. H.

American Review of Respiratory Disease 131:314-320, 1985.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medicine, University of Cincinnati, Cincinnati, OH.

OZONE-INDUCED CHANGES IN MUSCARINIC BRONCHIAL REACTIVITY BY DIFFERENT TESTING METHODS

We examined the effect of ozone (O_3) on muscarinic bronchial reactivity in the guinea pig and compared reactivity determined by two different routes of agonist delivery. Reactivity before and from 4h to 2 days after O_3 exposure (3.0 ppm, 2h) was determined by measuring specific airway resistance upon administration of intravenous acetylcholine and/or aerosolized methacholine challenge in 34 unanesthetized, spontaneously breathing animals. Before exposure, we observed more gradual and reproducible results to intravenous agonist. After exposure, hyperreactvity to parenteral agonist occurred consistently, but not to inhaled agonist. Hyperreactivity demonstrable by either route was similar in magnitude and time course within 14h of exposure. Two days later, hyperreactivity to inhaled agonist had remitted; that to intravenous drug persisted. Our results indicate that variability in the occurrence and time course of O_3-induced hyperreactivity to inhaled agonist may be a consequence of the technique employed. The consistent occurrence of hyperreactivity after O_3 to parenteral agonist suggests mechanisms other than airway mucosal hyperpermeability are responsible for this hyperreactivity.

Roum, J. H. and *Murlas, C.*

Journal of Applied Physiology 57(6):1783-1789, 1984.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and American Lung Association.

From the Departments of Medicine, Physiology and Biophysics, University of California, Irvine, and Department of Medicine, University of Cincinnati, Cincinnati, OH.

OZONE-INDUCED BRONCHIAL HYPERREACTIVITY IN GUINEA PIGS IS ABOLISHED BY BW 755C OR FPL 55712 BUT NOT BY INDOMETHACIN

We investigated the effects of BW 755C, an inhibitor of both the cyclooygenase and lipoxygenase pathways of arachidonic acid metabolism; FPL 55712, a selective antagonist of slow-reacting substance of anaphylaxis; and indomethacin, a cyclooxygenase inhibitor, on bronchial reactivity after ozone exposure. Guinea pigs in groups of 5 were treated with BW 755C (10 mg/kg given intravenously), FPL 55712 (5 mg/kg given intravenously), or indomethacin (30 mg/kg given intraperitoneally) and studied before and 30 min after a 15-min exposure to 3.0 ppm ozone. These animals were compared

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with a similarly exposed group that was untreated (n=10). Reactivity was determined by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion in unanesthetized, spontaneously breathing animals. Prior to ozone exposure, we found that drug treatment did not affect either SRaw or muscarinic reactivity. After exposure to 3.0 ppm, all untreated guinea pigs showed substantial muscarinic hyperreactivity. Indomethacin treatment did not inhibit this effect. Furthermore, in the indomethacin-treated animals, marked elevations occurred in SRaw after ozone. In contrast, no change in SRaw or muscarinic reactivity occurred after ozone in any animal treated with either BW 755C or FPL 55712. We conclude that ozone-induced bronchial hyperreactivity in the guinea pig rapidly develops after a brief, high-level exposure. This effect may be mediated in part by lipoxygenase products derived from lung arachidonic acid metabolism post-ozone.

Lee, H. K. and *Murlas, C.*

American Review of Respiratory Disease **132**:1005-1009, 1985.

Other support: National Heart, Lung and Blood Institute.

From the Departments of Medicine and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

U-60,257 INHIBITS O_3-INDUCED BRONCHIAL HYPERREACTIVITY IN THE GUINEA PIG

We studied the effect on ozone-induced airway hyperreactivity of U-60,257, a pyrroloprostacyclin shown to inhibit leukotriene C/D biosynthesis in vitro. A group of 5 guinea pigs was pretreated with U-60,257 (5 mg/kg IV) and studied before and 30 min after a 15 min exposure to 3.0 ppm ozone. These animals were compared to a similarly exposed group that was untreated (n=10). Reactivity was determined by measuring specific airway resistance (SRaw) upon intravenous acetylcholine infusion in unanesthetized, spontaneously breathing animals. We found that U-60,257 treatment prior to ozone exposure did not affect either SRaw or muscarinic reactivity. After exposure to 3.0 ppm, all untreated guinea pigs showed substantial muscarinic hyperreactivity. In contrast, no significant change in SRaw or muscarinic reactivity occurred after ozone in any animal pretreated with U-60,257. We conclude that the ozone-induced bronchial hyperreactivity in the guinea pig rapidly develops after a brief, high level exposure. This effect may be mediated in part by leukotrienes generated upon ozone exposure.

Murlas, C. and Lee, H.K.

Prostaglandins 30(4):563-568, 1985.

Other support: National Heart, Lung and Blood Institute.

From the Departments of Medicine and Environmental Health, University of Cincinnati School of Medicine, Cincinnati, OH.

INDOMETHACIN INHIBITS THE AIRWAY HYPERRESPONSIVENESS BUT NOT THE NEUTROPHIL INFLUX INDUCED BY OZONE IN DOGS

To determine whether oxygenation products of arachidonic acid may be involved in the airway hyperresponsiveness induced by ozone exposure, we studied whether

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ozone-induced hyperresponsiveness in dogs could be inhibited by the prostaglandin synthetase inhibitor, indomethacin. Airway responsiveness was assessed with dose-response curves of acetylcholine aerosol *versus* pulmonary resistance in 2 sets of experiments: in one set, 5 dogs were given no indomethacin treatment and were studied both before and after ozone exposure (3.0 ppm, 2 h); in another set, the same dogs were studied before indomethacin treatment or ozone exposure and then during treatment (1 mg/kg every 12 h for 4 days) both before and after ozone exposure. On each occasion, we also determined the number of neutrophils in biopsies of the airway epithelium. When the dogs were not treated with indomethacin, ozone caused a marked increase in responsiveness to acetylcholine and a marked increase in the number of neutrophils in the airway epithelium. When the dogs were given indomethacin, responsiveness was no different during treatment than before treatment, but more importantly, responsiveness did not increase significantly after they were exposed to ozone. Interestingly, indomethacin treatment did not affect either the baseline number of epithelial neutrophils before ozone exposure or the increase in the number of neutrophils after exposure. The results suggest that oxygenation products of arachidonic acid that are sensitive to inhibition by indomethacin play a role in ozone-induced hyperresponsiveness without affecting the influx of neutrophils.

O'Byrne, P. M., Walters, E. H., Aizawa, H., Fabbri, L. M., Holtzman, M. J., and *Nadel, J.*

American Review of Respiratory Disease 130:220-224, 1984.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

CONTROL OF NEUROTRANSMISSION BY PROSTAGLANDINS IN CANINE TRACHEALIS SMOOTH MUSCLE

Contractile responses of canine tracheal smooth muscle to electrical field stimulation diminished over a 2-h period of incubation. However, addition of indomethacin (10^{5}M) for a similar time not only prevented this inhibition of contractile response, but actually increased markedly the response to electrical field stimulation, suggesting that prostaglandins were responsible for the time-dependent inhibition. Measured prostaglandin E_2 increased in the tissue bath over 2-h in control tissues. Addition of prostaglandin E_2 to the tissue produced similar inhibition of contractile responses to electrical field stimulation in a concentration-dependent manner. In contrast, incubation alone, treatment with indomethacin or addition of prostaglandin E_2 had little, if any, effect on contractions induced by acetylcholine. We conclude that the release of prostaglandins from canine tracheal smooth muscle that occurs with time has a predominantly inhibitory effect on cholinergic neurotransmission at a prejunctional site.

Walters, E. H., O'Byrne, P. M., Fabbri, L. M., Graf, P. D., Holtzman, M. J. and *Nadel, J. A.*

Journal of Applied Physiology 57(1):129-134, 1984.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

AN ANTI-INFLAMMATORY DRUG (BW755C) INHIBITS AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE IN DOGS

To follow up our previous observation that airway hyperresponsiveness induced by ozone is linked to airway inflammation, we investigated the effect of BW755C, an anti-inflammatory drug, on ozone-induced hyperresponsiveness in dogs. Airway responsiveness was assessed with dose-response curves of acetylcholine aerosol *versus* pulmonary resistance in two sets of experiments. In one set (placebo treatment), five dogs were given only saline solution treatment and were studied before treatment or ozone exposure and then after treatment both before and after ozone (3.0 ppm, 2 hours); in another set (BW755C treatment), the same dogs were studied before BW755C treatment or ozone and then after treatment (10 mg/kg intravenously) both before and after ozone. When the dogs were not given BW755C, ozone induced a marked increase in airway responsiveness to acetylcholine. When the dogs were given BW755C, responsiveness was no different during treatment than before treatment but, more importantly, responsiveness did not increase significantly after ozone. It is concluded that BW755C markedly inhibits ozone-induced airway hyperresponsiveness in dogs, probably by inhibiting the formation of oxygenation products of arachidonic acid.

Fabbri, L. M., Aizawa, H., O'Byrne, P. M., Bethel, R. A., Walters, E. H., Holtzman, M. J., and *Nadel, J. A.*

Journal of Allergy and Clinical Immunology 76:162-166, 1985.

Other support: National Institutes of Health, Fisons Corporation, California Air Resources Board, and the Italian National Research Council.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

PREDOMINANT GENERATION OF 15-LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID BY EPITHELIAL CELLS FROM HUMAN TRACHEA

Epithelial cells of 99% purity and 92% viability were isolated from human tracheas obtained post mortem, and the cellular pathways for lipoxygenation of arachidonic acid were examined *in vitro*. The lipoxygenase metabolites were identified by comparison with synthetic standards during reversed-phase and straight-phase high pressure liquid chromatography, UV spectroscopy and gas chromatography/mass spectrometry. Epithelial cells incubated without arachidonic acid failed to generate detectable quantities of metabolites, while cells incubated with arachidonic acid at 1-50 μg/ml for 1-30 min invariably generated predominantly 15-lipoxygenase products, including 15-hydroxyicosatetraenoic acid (15-HETE), four isomers of 8,15-dihydroxyicosatetraenoic acid (two 8, 15 diHETES and two 8,15-leukotrienes), at least one isomer of 14,15-dihydroxyicosatetraenoic acid, and smaller amounts of 12-HETE and 8-HETE, but little or no detectable 5-HETE or 5,12-diHETEs. The capacity of epithelial cells from human pulmonary airway to selectively generate 15-lipoxygenase metabolites of arachidonic acid suggests a potential role for the products as mediators of airway epithelial function.

Hunter, J. A., Finkbeiner, W. E., *Nadel, J. A.*, Goetzl, E. J., and Holtzman, M. J.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

Proceedings of the National Academy of Sciences of the United States of America 82:4633-4637, 1985.

Other support: National Institutes of Health and Cystic Fibrosis Research Foundation.

From the Cardiovascular Research Institute; Howard Hughes Medical Institute; and Departments of Medicine, Pathology and Physiology, University of California, San Francisco.

LEUKOTRIENE B_4 INDUCES AIRWAY HYPERRESPONSIVENESS IN DOGS

The authors studied the effect of leukotriene B_4 aerosols on airway responsiveness to inhaled acetylcholine aerosols and on the cellular components and cyclooxyngenase metabolites in bronchoalveolar lavage fluid in dogs. Inhalation of leukotriene B_4 aerosols had no effect on resting total pulmonary resistance but increased airway responsiveness, and effect that was maximum in 3 h and that returned to control levels within 1 wk. Three hours after leukotriene B_4, the number of neutrophils and the concentration of thromboxane B_2 recovered in lavage fluid increased markedly. Pretreatment with the thromboxane synthase inhibitor OKY-046 prevented the increases in airway responsiveness and in thromboxane B_2 but did not alter neutrophil chemotaxis. Thus we speculate that leukotriene B_4 causes neutrophil chemotaxis and release of thomboxane B_2, which increases airway responsiveness.

O'Byrne, P. M., Leikauf, G. D., Aizawa, H., Bethel, R. A., Ueki, I. F., Holtzman, M. J., and *Nadel, J. A.*

Journal of Applied Physiology 596(6):1941-1946, 1985.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation, and the California Air Resources Board.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

PROSTAGLANDIN $F_{2\alpha}$ INCREASES RESPONSIVENESS OF PULMONARY AIRWAYS IN DOGS.

The authors studied the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on the responsiveness of pulmonary airways in dogs. Airway responsiveness was assessed by determining the bronchoconstrictor response to increasing concentrations of acetylcholine aerosol delivered to the airways. In each of five dogs, we determined responsiveness during treatment with physiologic saline, histamine, or $PGF_{2\alpha}$ aerosols. The doses of histamine and $PGF_{2\alpha}$ were determined by establishing the largest dose of each which could be given to the dog without causing bronchoconstriction (subthreshold doses). We found that airway responsiveness was not significantly different during histamine treatment than after saline; however, responsiveness increased during treatment with $PGF_{2\alpha}$. In addition, the hyperresponsiveness induced by $PGF_{2\alpha}$ was prevented by pretreatment with the ganglion blocking drug hexamethonium (5 mg/kg given intravenously). The results show that $PGF_{2\alpha}$ specifically increases the responsiveness of pulmonary airways in doses that do not cause bronchoconstriction, and suggest that the hyperresponsiveness involves a neural mechanism such as increased responsiveness of airway sensory nerves.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

O'Byrne, P. M., Aizawa, H., Bethel, R. A., Chung, K. F., *Nadel, J. A.*, and Holtzman, M.J.

Prostaglandins 28(4):537-543,1984.

Other support: National Heart, Lung and Blood Institute, the California Air Resources Board and Fisons Corporation.

From the Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco.

NEUTROPHIL DEPLETION INHIBITS AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE EXPOSURE

The authors studied whether ozone-induced hyperresponsiveness could be inhibited by neutrophil depletion in dogs. Responsiveness was assessed with dose-response curves of acetylcholine aerosol *versus* pulmonary resistance; depletion was assessed by counting neutrophils in venous blood and in biopsies of the airway epithelium. Responsiveness and neutrophil numbers were determined 5 days and 1 day before ozone and 1 h after ozone (3.0 ppm, 2 h) in 6 untreated dogs and in 6 dogs treated with hydroxyurea (200 mg/kg daily for 5 days starting 5 days before ozone). In untreated dogs, responsiveness and neutrophil numbers 5 days and 1 day before ozone did not change, but responsiveness and epithelial neutrophils increased markedly after ozone. In treated dogs, circulating neutrophils decreased from 8.9 ± 2.2 to 0.6 ± 0.01 X 10^3 per mm^3 (mean ± SEM), and responsiveness before ozone did not change. Furthermore, increases in responsiveness and epithelial neutrophils did not occur after ozone. Six wk after stopping hydroxyurea, responsiveness and epithelial neutrophils increased markedly after ozone. The results suggest that ozone-induced hyperresponsiveness may depend on the mobilization of neutrophils into the airways.

O'Byrne, P. M., Walters, E. H., Gold, B. D., Aizawa, H. A., Fabri, L. M., Alpert, S. E., *Nadel, J. A.*, and Holtzman, M. J.

American Review of Respiratory Disease 130:214-219, 1984.

Other support: National Heart, Lung and Blood Institute and Fisons Corporation.

From the Cardiovascular Research Institute, and Departments of Medicine and Physiology, University of California, San Francisco.

AIRWAY HYPERRESPONSIVENESS AND CHANGES IN CELL COUNTS IN BRONCHOALVEOLAR LAVAGE AFTER OZONE EXPOSURE IN DOGS

The authors studied whether airway hyperresponsiveness induced by ozone exposure is associated with changes in the numbers of different types of cells in bronchoalveolar lavage in dogs. Airway responsiveness to acetylcholine and the numbers of cells in lavage fluid were determined 1 wk before and then 1 h and 1 wk after 2-h exposures to filtered air and to ozone (3.0 ppm) in each of 5 dogs. Airway responsiveness and the numbers of cells in lavage fluid did not change after exposure to filtered air. By contrast, airway responsiveness increased markedly 1 h after exposure to ozone and returned to control levels 1 wk later. In addition, the numbers of neutrophils and of ciliated epithelial cells in lavage increased markedly 1 h after ozone and returned to control levels 1 wk later. Our previous study showed that airway hyperresponsiveness

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induced by ozone is associated with an influx of neutrophils into the most central airways; the present results suggest that the hyperresponsiveness is also accompanied by an influx of neutrophils into more distall airways and by desquamation of airway epithelial cells.

Fabri, L. M., *Nadel, J. A., et al.*

American Review of Respiratory Disease 129:288-291, 1984.

Other support: National Heart, Lung and Blood Institute, Fisons Corporation and the California Air Resources Board.

From the Cardiovascular Research Institute, Departments of Medicine and Physiology, University of California, San Francisco.

OCCURRENCE AND DISTRIBUTION OF REGULATORY PEPTIDES IN THE RESPIRATORY TRACT

A multimembered system of active regulatory peptides has been found in the respiratory tract. Studies on the neuroendocrine marker, neuron-specific enolase, indicate that there are still further types of mucosal endocrine cells and nerves whose products remain to be elucidated. This is not surprising in view of the rate of discovery of novel regulatory peptides by new biochemical methods and recombinant DNA technology. It is well known that the lung possesses functions in addition to mere respiration and these functions may well be regulated by the various regulatory peptides present in the respiratory tract. Many diseases of the lung are still poorly understood. The discovery of regulatory peptides involved in regulating mucosecretion, blood flow and muscle tone will undoubtedly contribute to the further understanding of these diseases.

The basic mechanism of tumor growth and the biology of tumor cells will also be better understood after further study of the active regulatory peptides, many of which play an important role as autocoids in the maintenance of tumor growth.

Polak, J. M. and Bloom, S. R.

Cancer Research 99:1-16, 1985.

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England.

BRONCHODILATOR RESPONSE DURING ACUTE VIRAL BRONCHIOLITIS IN INFANCY

Bronchodilator responsiveness was assessed by measuring specific respiratory conductance before and after inhalation of aerosolized bronchodilator in 50 infants who had acute bronchiolitis due to respiratory syncytial virus infection. Thirty percent of the infants showed an improvement in specific conductance. Responders could not be differentiated from nonresponders by family histories of atopy, eosinophil counts or immunoglobulin levels in blood and nasal secretions. Eighty-three percent of the families and 54% of the mothers of the infants were smokers. Babies of smoking mothers had lower specific conductances than did those of nonsmoking mothers but showed no differences in bronchodilator response. The clinical significance of this bronchodilator-responsive subgroup has yet to be defined.

Soto, M. E., Sly, P., Uren, E., *Taussig, L. M.*, and Landau, L.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

Pediatric Pulmonology 1(2):85-86, 1985.

Other support: National Institutes of Health.

From the Professional Department of Thoracic Medicine, Royal Children's Hospital, Melbourne, Australia.

UTILIZATION OF A PEROXIDASE ANTIPEROXIDASE COMPLEX IN AN ENZYME-LINKED IMMUNOSORBENT ASSAY OF ELASTIN-DERIVED PEPTIDES IN HUMAN PLASMA

Chronic obstructive pulmonary disease (COPD), a major cause of morbidity and death in the smoking population, develops insidiously over many years, and significant impairment of lung function usually occurs before the disease is diagnosed. Because lung elastin degradation appears to be a prerequisite for the development of the disease, immunologic detection of elastin-derived peptides in the blood might be an effective approach to the early detection and monitoring of the disease. We here report an improved enzyme-linked immunosorbent assay for elastin peptides using a peroxidase-antiperoxidase complex as the reporter group. The assay is sensitive to 2ng/ml elastin peptides. We show that for optimal, reproducible results the assay should be carried out at 16° C rather than at room temperature and that determinations should be made on plasma containing protease inhibitors rather than on serum. The levels of elastin-derived peptides appeared to remain relatively constant when multiple samples were taken during a 5- to 10-wk period from individual subjects. In addition, patients with COPD had elevated elastin peptide levels (127±47ng/ml), whereas normal smokers had values intermediate between the 2 groups (mean peptide levels of 76±42ng/ml) compared with levels in normal nonsmokers (58±17ng/ml). A small group of normal smokers (20%) had elevated peptide levels similar to those in the emphysema group and may represent that groups of smokers at risk of developing obstructive lung disease.

Kucich, U., *Weinbaum, G., et al.*

American Review of Respiratory Disease 131:700-713, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, Graduate Hospital, Albert Einstein Medical Center and Center for Oral Health Research, School of Dental Medicine, University of Pennsylvania, Philadelphia.

AMINO ACID DERIVED LATENT ISOCYANATES: IRREVERSIBLE INACTIVATION OF PORCINE PANCREATIC ELASTASE AND HUMAN LEUKOCYTE ELASTASE

Several amino acid derived azolides have been synthesized and investigated for their inhibitory activity toward human leukocyte elastase and porcine pancreatic elastase. The inhibitory activity was found to be dependent on the nature of the precursor amino acid ester. Thus, compounds derived from L-valine methyl ester 3, L-norvaline methyl ester 5, DL-norleucine methyl ester 9, and L-methionine methyl ester 10, were found to inhibit irreversibly both enzymes. Compound 10 was found to be a specific and selective inhibitor of human leukocyte elastase. In contrast to these, inhibitors derived from glycine methyl ester 1, D-valine methyl ester 4, and D-norvaline methyl ester 6 were

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found to be inactive. The results of the present study show that latent isocyanates derived from appropriate amino acids can serve as selective inhibitors of serine proteases and are of potential pharmacological value.

Groutas, W. C., *Weinbaum, G., et al.*

Journal of Medicinal Chemistry **28**(2):204-209, 1985.

Other support: American Chemical Society, American Lung Association and National Heart, Lung and Blood Institute.

From the Department of Chemistry, Wichita State University, Wichita, KS; the Graduate Hospital, Department of Medicine, Research Division, Philadelphia; the Department of Veterinary Biosciences, University of Illinois, Urbana; and the Department of Chemistry, University of Wisconsin, Eau Claire.

COLLAGENASE IN THE LOWER RESPIRATORY TRACT OF PATIENTS WITH ADULT RESPIRATORY DISTRESS SYNDROME

Collagenase activity in the bronchoalveolar lavage (BAL) of patients with adult respiratory distress syndrome (ARDS) was measured against Type I collagen (17 patients) and against Type III collagen (13 patients). Serine protease activity was also measured against Type III collagen (13 patients). Type I collagenase activity was detectable in 12 of 17 and Type III collagenase was detectable in 12 of 13 patients with ARDS. The 10 control subjects had no detectable Type I or III collagenase activity. Total and differential white cell counts were analyzed in the lavage fluid. Although the total counts did not differ between patients with ARDS and control subjects, the percentage of neutrophils was increased more than 25-fold and the percentage of macrophages was reduced almost 10-fold in the ARDS patients. Serial collagenase activity was followed in 1 ARDS survivor. In this patient Type III collagenase activity peaked before the Type I collagenase activity or serine protease activity reached their maximums. Both the last two enzyme activities paralleled the total recoverable cells in the BAL.

Christner, P., *Weinbaum, G., et al.*

American Review of Respiratory Disease **131**:690-695, 1985.

Other support: U. S. Public Health Service.

From the Division of Pulmonary and Critical Care Medicine, Albert Einstein Medical Center, New York; Temple University Health Science Center; the Department of Dental Histology, University of Pennsylvania; and the Division of Research, Department of Medicine, Graduate Hospital, Philadelphia.

SECRETION OF ELASTIN IN THE EMBRYONIC CHICK AORTA AS VISUALIZED BY IMMUNOELECTRON MICROSCOPY

Recently, significant advances have been made in characterizing the pathway of elastin biosynthesis from the biochemical point of view and a 70,000 dalton protein, designated tropoelastin, appears to be the primary translation product and soluble intermediate of the insoluble elastin. However, relatively little is known concerning the intracellular secretory pathway of tropoelastin. We previously developed an electron microscopic technique using elastin-specific antibody and ferritin-conjugated secondary

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antibody to identify intracellular elastin and to identify, provisionally, intracellular vesicles containing elastin. However, the method did not permit localization of elastin in other intracellular organelles. We now describe an improved post-embedding technique using the peroxidase-antiperoxidase method to detect the primary elastin antibody; we have localized elastin in both the endothelial and medial cells of the embryonic chick aorta. Specific staining was visualized in the cisternae of the endoplasmic reticulum, in the Golgi apparatus, and in vesicles forming on the trans side of the Golgi. Some of these smaller vesicles appeared to fuse, forming larger vesicles which may have a storage function. Both types of vesicles were seen fusing with the cell plasma membrane, suggesting that elastin is secreted by an exocytotic process. These results suggest that tropoelastin follows the classical pathway for protein secretion.

Damiano, V., Tsang, A. L., *Weinbaum, G.*, Christner, P., and Rosenbloom, J.

Collagen Related Research 4:153-164, 1984.

Other support: National Institutes of Health.

From the Center for Oral Health Research, School of Dental Medicine; the Department of Medicine, Graduate Hospital; and University of Pennsylvania, Philadelphia.

PROTEINASE INHIBITORY FUNCTION IN INFLAMMATORY LUNG DISEASE
I. ACUTE BACTERIAL PNEUMONIA

This study examines bronchial alveolar lavage (BAL) samples from a group of patients with acute bacterial pneumonia (n=13) and makes a comparison with a control group (N=5). The proteinase inhibitory capacity was examined and found to be composed primarily of alpha$_1$-proteinase inhibitor (PI; alpha$_1$-antitrypsin) and, to a lesser extent, bronchial mucosal inhibitor. Although the average PI concentration was elevated approximately 5-fold in the pneumonia group, its inhibitory function against elastase was decreased 15-fold when compared with that in the control group. The pneumonia group showed an increased concentration of immunologically identified elastin-derived peptides. Some of the BAL fluid from patients with pneumonia showed elastolytic activity against amorphous insoluble lung elastin. The majority of the elastase appears to be of neutrophil origin. Bronchial mucosal inhibitor is shown to be a component of both normal and pneumonia BAL fluids by both immunologic quantitation and by its resistance to perchloric acid inactivation. Compared with those from control subjects, BAL samples from patients with acute bacterial pneumonia showed a decreased proteinase inhibitor function and both increased elastolytic activity and elastin-derived peptide concentration.

Abrams, W. R., *Weinbaum, G. et al.*

American Review of Respiratory Disease 129:735-741, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Graduate Hospital, Department of Medicine, Research Division; Albert Einstein Medical Center, Department of Medicine, Pulmonary and Critical Care Divisions; Temple University Health Science Center, Pulmonary Disease Section, Philadelphia, and the Pulmonary Disease Department, Saga Medical School, Japan.

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NEUTROPHIL ELASTASE-RELEASING FACTORS IN BRONCHOALVEOLAR LAVAGE FROM PATIENTS WITH ADULT RESPIRATORY DISTRESS SYNDROME

Bronchoalveolar lavage fluid (BAL) was obtained from patients with adult respiratory distress syndrome (ARDS). Controls included BAL from normal subjects and from patients with sarcoidosis or pulmonary fibrosis. Neutrophil elastase measured immunologically was found in all BAL samples, but it was strikingly greater in BAL from patients with ARDS than in the BAL from normal subjects or patients with sarcoidosis. There was no significant difference in the neutrophil elastase antigen concentrations in BAL samples from patients with ARDS and those with pulmonary fibrosis. No elastolytic activity was found in either group. The alpha-1-antitrypsin and the bronchial mucus inhibitor were greater in BAL from patients with ARDS. There was a highly significant correlation between the alveolar-arterial oxygen tension difference and the neutrophil elastase concentration in BAL from the patients with ARDS. Kallikrein, prerekallikrein, factor XIa-like activity, and high molecular weight kininogen antigen were found in BAL of patients with ARDS, suggesting that the kallikrein-kinin cascade may be activated in the lungs of patients with ARDS. Kallikrein-like activity in the BAL from the patients with ARDS was significantly correlated with the number of neutrophils in the BAL, the neutrophil elastase concentration, and the ability of the BAL to release elastase from cytochalasin-B-treated neutrophils. There was no correlation between these variables and C5a concentration. These studies demonstrated an association between BAL neutrophil elastase and the clinical state of patients with ARDS.

Idell, S., *Weinbaum, G. et al.*

American Review of Respiratory Disease 132:1098-1105, 1985.

Other support: National Heart, Lung and Blood Institute, National Institutes of Health and American Heart Association, Pennsylvania Affiliate.

From the Pulmonary Disease and Hematology-Oncology Sections, Department of Medicine, and Thrombosis Research Center, Temple University Medical School; Pulmonary Disease Section, Albert Einstein Medical Center, Northern Division, Philadelphia.

OXIDANTS INCREASE PARACELLULAR PERMEABILITY IN A CULTURED EPITHELIAL CELL LINE.

Inflammation of epithelia is an important step in the pathophysiology of a wide variety of diseases. Because reactive oxygen metabolites are important effector molecules of acute inflammation, we examined the effect of oxidants on the barrier function of a cultured epithelium, Madin Darby Canine Kidney cells, by measuring the transepithelial electrical conductance, G_t, of monolayers grown on permeable supports. We found that H_2O_2, added directly or generated with glucose oxidase, increased G_t. Similar effects were observed with addition of xanthine and xanthine oxidase, a system which enzymatically generates superoxide radical O_2^-. The oxidant-induced increase in conductance was reversible if the exposure to oxidants was not prolonged ($t \leq 20$ min), and if the concentration of H_2O_2 was $< 5 \times 10^{-3}$ M. The increase in G_t suggested that oxidants increase the permeability of the paracellular pathway, a suggestion supported by an oxidant-induced increase in the permeability to ^{14}C-mannitol, which primarily crosses epithelia via the extracellular route.

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In addition to functional changes in the epithelial monolayer, oxidants changed the cell morphology; after H_2O_2 exposure, the cells tended to pull apart, most prominently at their basolateral surfaces. These changes were heterogeneous, with most areas showing no changes. Some of the morphologic changes could be reversed if the exposure to H_2O_2 was limited. We also observed a disruption of the normal pattern of the actin-cytoskeleton, particularly in the area of cell to cell junctions, as demonstrated by fluorescent staining of f-actin with rhodamine phallicidin.

These functional and structural findings indicate that oxidants increase the permeability of the paracellular pathway in a cultured epithelium. The changes can be reversible and are accompanied by alterations in organization of the cell cytoskeleton. These studies demonstrate the dynamic nature of the interaction between epithelial cells and oxygen metabolites.

Welsh, M. J., Shasby, D. M., and Husted, R. M.

Journal of Clinical Investigation 76:1155-1168, 1985.

Other support: National Institutes of Health, American Heart Association and Veterans Administration.

From the Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, and Veterans Administration Hospital, Iowa City.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

CHARACTERIZATION OF LIPOPROTEIN PARTICLES ISOLATED BY IMMUNOAFFINITY CHROMATOGRAPHY

Two populations of A-I-containing lipoprotein particles: A-I-containing lipoprotein with A-II (Lp (A-I with A-II)), and A-I containing lipoprotein without A-II (Lp (A-I without A-II)) have been isolated from plasma of 10 normolipidemic subjects by immunoaffinity chromatography and characterized. Both types of particles possess α-electrophoretic mobility and hydrated density in the range of plasma high-density lipoproteins (HDL). Lp (A-I without A-II) and Lp (A-I with A-II) are heterogeneous in size. Lp (A-I without A-II) comprised two distinct particle sizes with mean apparent molecular weight and Stokes diameter of 3.01 x 10^5, and 10.8 nm for Lp (A-I without A-II)$_1$, and 1.64 x 10^5, and 8.5 nm for Lp (A-I without A-II)$_2$. Lp (A-I with A-II) usually contained particles at least three distinct molecular sizes with mean apparent molecular weight and Stokes diameter of 2.28 x 10^6 and 9.6 nm for Lp (A-I with A-II)$_1$, 1.80 x 10^5 and 8.9 nm for Lp (A-I with A-II)$_2$, and 1.25 x 10^5 and 8.0 nm for Lp (A-I with II)$_3$. Apoproteins C, D and E, and lecithin:cholesterol acyltransferase (LCAT) were detected in both Lp (A-I without A-II) and Lp (A-I with A-II) with most of the apoprotein D, and E, and LCAT (EC 2.3.1.43) in Lp (A-I with A-II) particles. Lp (A-I without A-II) had slightly higher lipid/protein ratio than Lp (A-I with A-II). Lp (A-I with A-II) had an A-I/A-II molar ratio of approximately 2:1. The percentage of plasma A-I associated with Lp (A-I without A-II) was highly correlated with the A-I/A-II ratio of plasma (r = 0.96, n = 10). The variation in A-I/A-II ratio of HDL density subfractions therefore reflects different proportions of two discrete types of particles: particles containing A-I and A-II in a nearly constant ratio and particles containing A-I but no A-II. Each type of particle is heterogeneous in size and in apoprotein composition.

Cheung, M. C. and *Albers, J. J.*

The Journal of Biological Chemistry 259(19):12201-12209, 1984.

Other support: National Institutes of Health and the American Cancer Society.

From the Department of Medicine, University of Washington, Seattle.

PLATELET-DERIVED GROWTH FACTOR AND MALIGNANT TRANSFORMATION

Studies on human platelet-derived growth factor (PDGF) described in this paper represent the collective effort of several teams of investigators over the past 10 years. These studies produced an abundance of information concerning the nature and structure of PDGF, its role in cell growth and its diverse functions affecting cell migration, metabolic processes and receptor modulation. This work also led to an important discovery linking this potent mitogen to the transforming protein of the simian sarcoma virus, providing a basis for the understanding of the processes involved in transformation induced by the SSV *onc* gene. Specificially, the findings described here provided the missing link for the understanding of the mechanism of cell transformation induced by the *onc* gene of the simian sarcoma virus. This *onc* gene, *v-sis*, was shown to encode a PDGF-like polypeptide which is a potent mitogen for fibroblasts, arterial smooth muscle

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cells and glial cells. Activation of *sis* transcription may cause the sustained abnormal proliferation of cells responsive to the mitogenic effects of the PDGF-like molecule. Overall, these findings are consistent with the suggestion that *sis* activation might be involved in the process leading normal cells of certain types towards malignancy.

Antoniades, H.N.

Biochemical Pharmacology 3(18):2823-2828, 1984.

Other support: U.S. Public Health Service and the American Cancer Society.

From the Center for Blood Research and the Department of Nutrition, Harvard School of Public Health, Boston.

SYNTHESIS AND SECRETION OF PROTEINS RESEMBLING PLATELET-DERIVED GROWTH FACTOR BY HUMAN GLIOBLASTOMA AND FIBROSARCOMA CELLS IN CULTURE

Immunoprecipitation of proteins extracted from metabolically labeled human glioblastoma and fibrosarcoma cells with antiserum to platelet-derived growth factor (PDGF) showed that these cells express and secrete proteins that are recognized specifically by the antiserum. The molecular masses of immunoprecipitated proteins in the lysates of the malignant cells ranged from 16 kDa to 140 kDa. Both cell lines secreted a 31-kDa polypeptide with structural, immunological and biological properties similar to those of human PDGF. These cell lines were shown to synthesize a 4.4-kb mRNA that contained sequences from all the six currently identified exons of the human c-*sis* gene. These data suggest that the PDGF-like proteins in the two mesenchyme-derived transformed cells are encoded at least in part by the c-*sis* locus.

In this report we describe the intracellular synthesis of PDGF-like proteins in cultures of human glioblastoma and fibrosarcoma cells and correlate the presence of these proteins with a c-*sis* mRNA species transcribed by the human c-*sis* locus. The intracellular PDGF-like proteins appear to be precursor and processed molecules of a major secreted protein of 31 kDa that is structurally, immunologically and functionally similar to human PDGF.

Pantazis, P., Pelicci, P.G., Dalla-Favera, R., and *Antoniades, H.N.*

Proceedings of the National Academy of Sciences of the United States of America 82:2404-2408, 1985.

Other support: National Institutes of Health and the American Cancer Society.

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston, and the Department of Pathology, Kaplan [illegible] Center, New York University School of Medicine, New York.

PLATELET-DERIVED GROWTH FACTOR POLYPEPTIDES IN HUMAN MEGAKARYOBLASTIC-LIKE CELL LINES

Novel cell lines derived from human peripheral blood were shown to synthesize polypeptides recognized by specific antisera to human platelet-derived growth factor (PDGF). Metabolically labeled intracellular polypeptides were immunoprecipitated by PDGF antiserum and were analyzed by SDS-polyacrylamide gel electrophoresis. The

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molecular masses of the intracellular forms of PDGF-like polypeptides in these cell lines ranged from 12,000 to 48,000 daltons (12-48 kD), suggesting the presence of PDGF precursor and processed molecules. Metabolically labeled polypeptides immunoprecipitated by PDGF antisera were also recognized in the conditioned media of one of the cell lines obtained from a leukemic patient. The molecular masses of the secreted PDGF-like polypeptides were 12 kD, 31 kD, and 46 kD. Upon reduction, the 31-kD polypeptide was reduced to 16 kD, suggesting that it represents a disulfide-linked dimer similar to that described for PDGF.

Pantazis, P., Morgan, D.A., Brodsky, I., and *Antoniades, H.N.*

In: *CANCER CELLS 3/Growth Factors and Transformation*: Cold Spring Harbor Laboratory, 1985, pp. 153-157.

Other support: National Institutes of Health and American Cancer Society.

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston, and the Department of Hematology/Oncology, Hahnemann University, Philadelphia.

EFFECT OF CALCIUM ON THE STABILITY OF THE PLATELET MEMBRANE GLYCOPROTEIN IIb-IIIa COMPLEX

Platelet membrane glycoproteins IIb and IIIa form a Ca^{2+}-dependent heterodimer complex that contains binding sites for fibrinogen, von Willebrand factor, and fibronectin following platelet stimulation. The authors have studied the effect of Ca^{2+} on the stability of the IIb-IIIa complex using a IIb-IIIa complex specific monoclonal antibody A_2A_9 to detect the presence of the complexes. Soluble IIb and IIIa interacted with A_2A_9-Sepharose only in the presence of Ca^{2+} with 50% IIb-IIIa binding requiring 0.4 μM Ca^{2+}. In contrast, at 25°C the binding of radioiodine labelled A_2A_9 to intact unstimulated platelets suspended in buffers containing EDTA or ethylene glycol bis(β-aminoethyl ether) *N,N,N',N'*,-tetracetic acid was independent of the presence of Ca^{2+}. However, the effect of Ca^{2+} chelators on ^{125}I-A_2A_9 binding varied with temperature. At 37°C, ^{125}I-A_2A_9 binding to intact platelets became Ca^{2+}-dependent with 50% binding requiring 0.4 μM Ca^{2+}. This effect of temperature was not due to a change in platelet membrane fluidity because enrichment or depletion of platelet membrane cholesterol did not influence antibody binding. Also, ^{125}I-A_2A_9 binding to intact platelets at 25° C became Ca^{2+}-dependent when the pH was increased above 7.4. At 1 nM Ca^{2+} and 25°C, 50% antibody binding occurred at pH 9.0. These studies demonstrate that Ca^{2+}- dependent IIb-IIIa complexes are present on unstimulated platelets and that the Ca^{2+} binding sites responsible for the stability of these complexes are located on the external platelet surface. These experiments also suggest that changes in platelet cytosolic Ca^{2+} do not regulate the formation of IIb-IIIa complexes.

Brass, L. F., Shattil, S. J., Kunick, T. J., and *Bennett, J. S.*

The Journal of Biological Chemistry 260(13):7875-7881, 1985.

Other support: National Institutes of Health and the American Heart Association.

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From the Hematology-Oncology Section and the Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, and the Blood Center of Southeastern Wisconsin, Milwaukee.

THE TETRAPEPTIDE ANALOGUE OF THE CELL ATTACHMENT SITE OF FIBRONECTIN INHIBITS PLATELET AGGREGATION AND FIBRINOGEN BINDING TO ACTIVATED PLATELETS

Fibrinogen binding to receptors on activated platelets is a prerequisite for platelet aggregation. However, the regions of fibrinogen interacting with these receptors have not been completely characterized. Fibronectin also binds to platelet fibrinogen receptors. Moreover, the amino acid sequence Arg-Gly-Asp-Ser, corresponding to the cell attachment site of fibronectin, is located near the carboxyl-terminal region of the α-chain of fibrinogen. The researchers have examined the ability of this tetrapeptide to inhibit platelet aggregation and fibrinogen binding to activated platelets. Arg-Gly-Asp-Ser, but not the peptide Arg-Gly-Tyr-Ser-Leu-Gly, inhibited platelet aggregation stimulated by ADP, collagen, and γ-thrombin without inhibiting platelet shape change or secretion. At a concentration of 60-80 μM, Arg-Gly-Asp-Ser inhibited the aggregation of ADP-stimulated gel-filtered platelets ≈ 50%. Arg- Gly-Asp-Ser, but not Arg-Gly-Tyr-Ser-Leu-Gly, also inhibited fibrinogen binding to ADP-stimulated platelets. This inhibition was competitive with a K_i of about 25 μM but was incomplete even at higher tetrapeptide concentrations, indicating that Arg-Gly-Asp-Ser is a partial competitive inhibitor of fibrinogen binding. These data suggest that a region near the carboxyl-terminus of the α-chain of fibrinogen interacts with the fibrinogen receptor on activated platelets. The data also support the concept that the sequence Arg-Gly-Asp-Ser has been conserved for use in a variety of cellular adhesive processes.

Gartner, T. K. and *Bennett, J. S.*

The Journal of Biological Chemistry 260(22):11891-11894, 1985.

Other support: National Institutes of Health.

From the Department of Biology, Memphis State University, Memphis, and the Hematology-Oncology Section and Cancer Center, University of Pennsylvania School of Medicine, Philadelphia.

CARDIAC INOTROPIC RESPONSE OF A NEW β-1-AGONIST (TA-064) WITH LOW SARCOLEMMAL ADENYLATE CYCLASE ACTIVATION

These experiments were concerned with an investigation of the effect of a new selective β-1-adrenergic compound, TA-064, on the function of purified sarcolemmal vesicles prepared from perfused canine heart preparations. Special emphasis was placed on the effect of TA-064 on adenylate cyclase activity, on ATP-dependent Ca^{++} uptake and on phosphorylation of purified sarcolemma (SL). Perfusion was carried out in a supported canine heart preparation with a perfluorochemical (FC-43) as perfusate. TA-064 (317 μg) was injected into the left atrium. As previously reported, this resulted in a marked positive inotropic and chronotropic effect which was abolished by propranolol. Purity of the SL preparation was confirmed with marker enzymes for mitochondria, sarcoplasmic reticulum and SL. In addition, Na^+Ca^{++}-exchange characteristic for SL was determined. Sidedness of the vesicles was ascertained by means

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of adenylate cyclase activity using SL preparations with and without alamethicin. TA-064, as compared to 1-isoproterenol, exerted little effect on adenylate cyclase activity. The addition of TA-064 either to the perfused whole heart preparation or to prepared SL vesicles resulted in small elevations of adenylate cyclase activity relative to 1-isoproterenol. TA-064 increased ATP-dependent Ca^{++} uptake and phosphorylation. The relative minor response of adenylate cyclase to TA-064 was apparently sufficient to elevate both myocardial contractility and phosphorylation and Ca^{++} dependent ATPase activity. Therefore, TA-064 belongs together with prenalterol in a group of β-1-agonists with full inotropic activity but relatively diminished capacity to activate adenylate cyclase. The pharmacologic and clinical implications of these findings were discussed.

Bing, R. J. et al.

Current Therapeutic Research 36(6):1127-1144, 1984.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

CARDIAC PERFUSION, PAST AND PRESENT

This paper was presented at a symposium which was devoted in part to the role of lipids in relation to cardiac metabolism and function. The work of pioneers in this area, including Tigerstedt, Ludwig, Lamgendorf, Wild, and Ringer, is noted. These early results were dwelt upon, according to the author, because they determined the future course of cardiac metabolism. Determination of the nutrition of the human heart in situ was a direct and logical extension of their work. More recently the course of cardiac metabolism has been influenced by biochemistry and biophysics by separation of subcellular particles and organelles. Renewed stimulation for the study of the isolated mammalian heart has come from the laboratory of Neely *et al.*, whose perfusion technique has been particularly useful in the understanding of the regulation of glycolysis in the ischemic and anoxic myocardium. Neely's perfusion studies were primarily carried out on the rat heart. This has limited the options for the study of glycolytic intermediates and glycolytic flux, excluding determinations of high energy phosphates, which necessitate specimens of greater weight. The use of perfluorochemicals has now made it possible to perfuse larger mammalian hearts using Neely's working heart preparation. The remainder of this discussion is concerned with hemodynamic and metabolic data on the different types of the failing rabbit heart perfused *in vitro* with Fluosol-43. The effect of TA-064, a new positive inotropic agent, is briefly mentioned. In addition, this paper deals with a new approach to the problem of coronary spasm, using the isolated heart perfused with Fluosol-43 oxygenated with a bubble oxygenator.

Bing, R. J.

In: Ferrari, R., Katz, A., Shug, A., and Visioli, O. (eds.): *Myocardial Ischemia and Lipid Metabolism*. New York: Plenum Publishing Corporation, 1984, pp. 267-281.

Other support: Hoover Foundation.

From the Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

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EFFECT OF INTACT ENDOTHELIUM AGAINST PLATELET-INDUCED CORONARY ARTERY SPASM IN ISOLATED RABBIT HEARTS

Effects of collagen-activated washed rabbit platelets on coronary arteries with and without intact endothelium were studied in a supported rabbit heart preparation using a perfluorocarbon (FC-43) as perfusate. The vascular diameter of obtuse marginal coronary arteries was determined by means of gated color arteriography (injection of patent blue dye). Endothelial denudation of the obtuse marginal artery was accomplished by scraping the lumen with a roughened plastic tubing. Injection of washed platelets (10 ml with about 500,000 platelets/μl) not treated with collagen failed to constrict coronary arteries either with intact or denuded endothelium. In contrast, injection of platelet suspension immediately after activation with collagen caused vasoconstriction of denuded obtuse marginal coronary arteries in 10 of 14 cases. In 6 preparations, occlusion was complete, lasting up to 16 minutes. In arteries with intact endothelium, no coronary vasoconstriction occurred. In hearts with coronary artery spasm, total coronary vascular resistance increased significantly. This study furnishes additional evidence that endothelial lesions are a contributory factor for large coronary artery spasm and that endothelial cells possess a protective function against vasoconstrictor substances released from aggregating platelets.

Bing, R. J., et al.

American Journal of Cardiology 55:1596-1600, 1985

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE EFFECT OF NIMODIPINE, A CALCIUM ANTAGONIST, ON INTRACORTICAL ARTERIOLES IN THE CAT BRAIN

The purpose of this study was to test the effect of the calcium antagonist nimodipinine on the intracortical arterioles and on cerebral blood flow in the anesthetized cat. Arteriolar diameter, red cell velocity and regional cerebral blood flows were determined by a technique employing transillumination and high speed cinematography. Global cerebral blood flow was measured using the microsphere technique. After a five-minute infusion of nimodipine (0.625 μg/kg), there was a significant increase in cortical arteriolar diameter ($p < 0.001$) and in regional blood flow ($p < 0.01$). Red cell velocity increased ($p < 0.01$). Global cerebral blood flow increased ($p < 0.02$) while mean arterial blood pressure declined ($p < 0.05$). Therefore, direct observations confirm that nimodipine dilates intracortical arterioles and increases red cell velocity in these vessels.

Schmidli, J., Santillan, G. G., Saeed, M., Palmieri, D., and *Bing, R. J.*

Current Therapeutic Research 38(1):94-103, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

BETA-1-ADRENOCEPTOR AGONISTS WITH LOW ADENYLATE CYCLASE ACTIVATION — THEORETICAL AND CLINICAL IMPLICATIONS

Several partial beta-agonists such as TA-064, prenalterol, ICI89,963, and ICI119,033 possess pronounced positive inotropic effects, but induce only limited activation of myocardial adenylate cyclase; this results in low cyclic AMP levels. Partial agonists, as compared to isoproterenol, need high receptor occupancy for maximal physiological response and demonstrate inadequate coupling between receptor and adenylate cyclase. However, the biochemical mechanisms which generate the final biological effects originating from cyclic AMP are tissue-specific, and for that reason, can maintain a pronounced positive inotropic action. Clinical data with TA-064 and prenalterol have established positive inotropic effects without significant changes in heart rate. Partial beta-agonists with diminished cyclic AMP may be less arrhythmogenic than full beta-1-agonists. The clinical importance of partial beta-1-agonists remains to be established, particularly with relevance to long-term exposure and inherent beta blockade.

Chemnitius, J. M. and *Bing, R. J.*

Canadian Journal of Cardiology 1(3):186-190, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

CRYSTALLOID AND PERFLUOROCHEMICAL PERFUSATES IN AN ISOLATED WORKING RABBIT HEART PREPARATION

Krebs-Henseleit buffer (KH) and a perfluorochemical (FC-43) were compared as perfusates in an isolated working rabbit heart preparation. Both perfusates were oxygenated in an identical manner using an infant bubble oxygenator. After 60 min of perfusion, no difference could be detected in the ratio of wet to dry heart weight between KH- and FC-43-perfused hearts (KH, 6.25 ± 0.3; FC-43, 5.99 ± 0.20). Left ventricular systolic pressure, maximal rate of left ventricular pressure rise, mean aortic systolic pressure, cardiac output, aortic flow, left ventricular power, and myocardial O_2 consumption (MVo_2) were significantly higher in FC-43-perfused hearts throughout the time of perfusion. However, there were no differences in resistance to cardiac output and heart rate. In KH- and FC-43-perfused hearts, MVo_2 and left ventricular power were closely correlated (KH, $r = 0.793$; FC-43, $r = 0.831$). Significantly higher coronary flow of KH-perfused hearts could be attributed to the lower viscosity of KH (1.05 Pa-s) compared with FC-43 (1.91 Pa-s) compared with FC-43 (1.91 Pa-s). Increased O_2 extraction during KH perfusion could not compensate for low O_2-carrying capacity of KH buffer (345 compared with 705 nmol O_2 ml^{-1} in FC-43 emulsion). A postischemic increase of coronary flow was observed only in FC-43-perfused hearts (28%). These results demonstrate a different response of perfused heart preparations to FC-43 and KH buffer.

Chemnitius, J. M., Burger, W., and *Bing, R. J.*

American Journal of Physiology 249(Heart Circ. Physiol.):H285-H292, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

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From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

A NEW METHOD FOR THE VISUALIZATION OF SUBEPICARDIAL CORONARY ARTERIES IN SMALL ISOLATED MAMMALIAN HEARTS

A model is described which permits direct visualization of large coronary arteries in a supported modified perfused heart preparation, using a perfluorochemical (FC-43) as perfusate. Filling of a large coronary artery with Patent Blue Dye is recorded by gated photography (color arteriography). The technique is applicable to the study of reactivity (spasm) of coronary arteries in hearts of small and large animals (rats, rabbits, dogs). The technique has the following advantages: preservation of vascular endothelium, adequate oxygenation, avoidance of major surgical intervention to implant sensors for the detection of changes in coronary diameter, quantitative evaluation of time-dependent changes in geometry of large coronary arteries, and simultaneous measurements of large coronary vessel and total coronary vascular resistance.

Burger, W., Chemnitius, J. M., and *Bing, R. J.*

Proceedings of the Society for Experimental Biology and Medicine 178:309-312, 1985

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE EFFECT OF CARDIOPULMONARY BYPASS ON CEREBRAL BLOOD FLOW

These experiments deal with the effect of total cardiopulmonary bypass on global and regional cerebral blood flow. Global cerebral blood flow was determined with radioactive microspheres, regional cerebral flow by direct observation through transillumination and high-speed cinematography. The latter permitted observations of changes in caliber of cerebral arterioles and of red cell velocity. Arterial blood gas tension and pH were continously monitored. Measurements were carried out prior to and immediately following cardiopulmonary bypass. Studies on global cerebral flows showed that during cardiopulmonary bypass, cerebral blood flow rose and cerebral vascular resistance declined while blood pressure did not change significantly. During bypass, cerebral blood flow and blood pressure in animals treated as a group became interdependent and showed direct proportionality. Direct observations of the cortical microvasculature by means of high-speed cinematography revealed dilatation of cerebral cortical arterioles. Even though blood pressure remained unchanged, the diameter of cortical arterioles increased. Volume flow calculated from red cell velocity and arteriolar diameter showed a tendency of arteriolar flow to increase. The condition is reminiscent of the "luxury perfusion symdrome" (overabundant cerebral flow relative to metabolic needs of cerebral tissue).

Santillan, G. C., Chemnitius, J. M., and *Bing, R. J.*

Brain Research 345:1-9, 1985.

Other support: Hoover Foundation and the Charles A. Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

A MODEL FOR THE STUDY OF CORONARY SPASM INDUCED CHANGES IN CARDIAC METABOLISM

A model is described which permits the study of localized and generalized arterial spasm in the intact working perfused rabbit heart with a perfluorochemical (FC-43) as perfusate. Coronary arteries were visualized by intraatrial injection of Patent Blue Dye with gated photography. Localized spasm resulted from topical spray of histamine (40 μmols) on the epicardial surface overlying an obtuse marginal artery. Before and following topical administration of histamine, regional coronary flow was determined using radioisotope-labeled microspheres. Generalized arterial spasm was initiated by intraatrial injection of histamine (10 μmols). After *topical administration*, obtuse marginal artery diameter decreased by 57%; large vessel resistance rose 32-fold; 20% rise of total coronary resistance resulted in a slight reduction of total coronary flow (16%). Heart rate, cardiac output, dP/dt_{max} and myocardial oxygen consumption did not change. However, regional coronary flow in the myocardium supplied by the affected artery diminished 21%, resulting in ischemic changes in redox pairs. After *intraatrial injection* of histamine, changes were more pronounced. Obtuse marginal artery diameter declined by 88%, resulting in 3300-fold rise of large vessel resistance. Total coronary resistance increased 150%, coronary flow and cardiac output diminished (56% and 24%). Both heart rate and dP/dt_{max} increased (16% and 17%). Generalized coronary spasm after intraatrial histamine injection resulted in several metabolic effects: Myocardial oxygen consumption (-48%); ATP (-29%); creatine phosphate (-34%); redox ratios, α-glycerophosphate/dihydroxyacetone phosphate and lactate/pyruvate, increased by 449% and 114%, respectively. The findings illustrate that localized and general coronary spasms can be produced and quantitated in a working heart model.

Burger, W., Chemnitius, J. M., Metz, M. Z., and *Bing, R. J.*

Journal of Molecular Cell Cardiology 17:917-930, 1985.

Other support: Hoover Foundation.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE EFFECT OF ISCHEMIA AND REPERFUSION ON SARCOLEMMAL FUNCTION IN PERFUSED CANINE HEARTS

This report deals with the effect of ischemia and reperfusion on purified sarcolemma obtained from canine myocardium of perfused heart preparation. Perfusion was carried out with a perfluorochemical (FC-43). Ischemia was produced by intermittent total clamping of inflow and outflow followed by reflow until the decrease in dp/dt_{max} had become stabile. Purity of sarcolemmal vesicles was ascertained with marker enzymes: succinate cytochrome c reductase (for mitochondria), K^+-stimulated p-nitrophenylphosphate (K^+-pNPPase), (Na^+/K^+) ATPase and adenylate cyclase (for SL). In addition Na^+/Ca^{++}-exchange characteristics for SL were determined. Sidedness of vesicles was ascertained by means of adenylate cyclase activity using sarcolemmal preparations treated and untreated with alamethicin. Emphasis was placed on ATP-dependent Ca^{++} uptake, phosphorylation of sarcolemmal vesicles and yield of SL proteins. Ischemia and reperfusion resulted in a significant reduction in adenylate

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cyclase activity. This decline was significant following ischemia and reperfusion. The yield of protein recovered from SL vesicles from ischemic-reperfused heart preparations was also significantly decreased. Both initial rate of ATP-dependent Ca^{++} uptake and maximal Ca^{++} uptake fell significantly following ischemia and reperfusion. The initial rate of phosphorylation also dropped significantly. These disturbances in SL Ca^{++} transport following ischemia and reperfusion are probably a part of the general deficit in Ca^{++} translocation.

Chemnitius, J. M., Sasaki, Y., Burger, W., and *Bing, R. J.*

Journal of Molecular and Cellular Cardiology 17(12):1139-1150, 1985.

Other support: Hoover Foundation and the Lindbergh Fund.

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

CLEAVAGE OF HUMAN HIGH MOLECULAR WEIGHT KININOGEN BY FACTOR XIa *IN VITRO*

We have recently demonstrated that human high molecular weight kininogen (HMWK) is a pro-cofactor that is cleaved by kallikrein to yield a two-chain cofactor (HMWKa) and the nanopeptide bradykinin. This proteolysis enhances its association with an activating surface, an event necessary for expression of its cofactor activity. We now report that factor XIa is capable of hydrolyzing HMWK and releasing bradykinin in a purified system as well as cleaving and inactivating HMWK in a plasma environment during the contact-activation process. The profile of proteolysis differs from that produced by kallikrein and by factor XIIa in that the first cleavage by factor XIa yield 75- and 45-kDa polypeptides, whereas both factor XIIa and kallikrein initially produce 65- and 56-kDa species. Further proteolysis by all three enzymes eventually produces similar heavy chains (M_r = 65,000) and light chains (45,000). However, the amount of factor XIa generated in plasma during contact-activation further degrades the light chain of HMWK, eventually destroying its coagulant activity. Furthermore, in a purified system, enhancement of the degradation of HMWK coagulant activity by factor XIa was achieved when kallikrein was included in the incubation mixture, suggesting that the preferred substrate for factor XIa is the active form of HMWK (HMWKa) and not the pro-cofactor. These data suggest that factor XIa has the potential to act as a regulator of contact-activated coagulation by virtue of its ability to destroy the cofactor function of HMWK after its generation by either kallikrein, factor XIIa, or to a lesser extent, factor XIa itself.

Scott, C. F., Silver, L. D., Purdon, A. D., and *Colman, R. W.*

The Journal of Biological Chemistry 260(19):10856-10863, 1985.

Other support: National Institutes of Health.

From the Thrombosis Research Center and the Hematology/Oncology Section of the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

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HUMAN PLASMA KALLIKREIN AND C1 INHIBITOR FORM A COMPLEX POSSESSING AN EPITOPE THAT IS NOT DETECTABLE ON THE PARENT MOLECULES: DEMONSTRATION USING A MONOCLONAL ANTIBODY

The inactivation of human plasma kallikrein (EC 3.4.21.8) by the inhibitor of activated complement component 1 (C1 inhibitor) induces the formation of a 1:1 stoichiometric kallikrein-C1 inhibitor complex and a proteolytically modified form of C1 inhibitor. We have produced a monoclonal antibody that recognizes the kallikrein-C1 inhibitor complex as well as modified C1 inhibitor, but fails to react with virgin C1 inhibitor or native plasma kallikrein. This observation constitutes an unequivocal demonstration that the reaction between plasma kallikrein and C1 inhibitor leads to the emergence of an epitope that is undetectable on the parent enzyme and inhibitor molecules.

Agostini, A. de, Schapiro, M., Wachtfogel, Y. T., *Colman, R.W.*, and Carrel, S.

Proceedings of the National Academy of Sciences of the United States of America 82:5190-5193, 1985.

Other support: Swiss National Science Foundation and U.S. National Institutes of Health.

From the Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Thrombosis Research Center and Hematology/Oncology Section, the Department of Medicine, Temple University School of Medicine, Philadelphia; and the Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland.

PLASMA KALLIKREIN AND PRORENIN IN PATIENTS WITH HEREDITARY ANGIOEDEMA

Recent evidence indicates that plasma kallikrein is activated during acute attacks of hereditary angioedema. Plasma kallikrein is known to convert inactive renin, or prorenin, into an active proteolytic enzyme in plasma exposed to acid or low temperatures as well as in purified systems. To establish whether plasma kallikrein could activate prorenin under physiologic or pathologic conditions, prorenin to renin conversion was assessed at neutral pH in plasma deficient in C1 inhibitor (hereditary angioedema). In these plasma samples lacking the two major inhibitors of kallikrein and possessing ≤ 10% of the inhibitory activity of normal plasma, prorenin was not converted to an active enzyme despite conditions under which prekallikrein was completely activated to plasma kallikrein and despite normal prorenin concentrations and activability.

Purdon, A. D., Schapira, M., Agostini, A. de and *Colman, R. W.*

The Journal of Laboratory and Clinical Medicine 105(6):694-699, 1985.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Thrombosis Research Center and Hematology/Oncology Section of the Department of Medicine, Temple University School of Medicine, Philadelphia, and the Division of Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

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PLATELET C1 INHIBITOR: A SECRETED ALPHA-GRANULE PROTEIN

In order to characterize which proteins of the contact phase of coagulation interact with platelets, human platelets were studied immunochemically and functionally to determine if they contain C1 inhibitor. By means of monospecific antibody to C1 inhibitor, a competitive enzyme-linked immunosorbent assay (CELISA) was developed to measure directly platelet C1 inhibitor. With the CELISA, from 33 to 115 ng of C1 inhibitor antigen per 10^8 platelets from 15 normal donors was quantified in lysates of washed human platelets solubilized in nonionic detergent. The mean concentration in 10^8 platelets was 62±33 ng (SD). Plasma C1 inhibitor either in the platelet suspension medium or on the surface of the platelets could account for only from 6.5 to 16% of the total antigen measured in the solubilized platelets. Upon functional studies, platelets contained 84±36 ng (SD) of C1 inhibitor activity in 10^8 platelets. As assessed by the CELISA, platelet C1 inhibitor antigen was immunochemically identical to plasma and purified C1 inhibitor. In contrast, the mean concentration of platelet C1 inhibitor antigen in platelets from four patients with classical hereditary angioedema was 8.3 ng/10^8 platelets (range 5.3 to 11.3 ng/10^8 platelets). 25 % and 31% of the total platelet C1 inhibitor was secreted without cell lysis from normal platelets after exposure to collagen (20 μg/ml) and thrombin (1U/ml), respectively, and this secretion was blocked by metabolic inhibitors. Platelet subcellular fractionation showed that platelet C1 inhibitor resided mostly in alpha-granules, similar to the location of platelet fibrinogen. Thus, human platelets contained C1 inhibitor that became available by platelet secretion. The identification of platelet C1 inhibitor suggests that platelets may modulate the activation of the proteins of early blood coagulation and the classical complement pathways.

Schmaier, A. H., Smith, P. M. and *Colman, R. W.*

Journal of Clinical Investigation 75:242-250, 1985.

Other support: National Heart, Lung and Blood Institute; a Temple University Biomedical Research Support Grant; American Heart Association; and a Specialized Centers of Research grant.

From the Hematology/Oncology Section, Department of Medicine, Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

EFFECT OF HEPARIN ON THE INACTIVATION RATE OF HUMAN ACTIVATED FACTOR XII BY ANTITHROMBIN III

Human antithrombin III (ATIII) is a plasma inhibitor of several serine proteases of the blood coagulation system. Previous investigations have reported that the presence of heparin has a multifold accelerating effect on the inhibition of factor XIIa and XIIf, the active species derived from factor XII. Recent studies from our laboratories have confirmed that ATIII inactivates factor XIIa and factor XIIf, but only contributes 2% to 3% to the inhibition of activated factor XII species in plasma. The major inhibitor is C1 inhibitor. Therefore, we have reexamined the heparin effect on the rate of inhibition of factor XIIa and factor XIIf in purified systems. We also have studied the effect of heparin on the inactivation of both factor XII-derived active species by various plasmas. Using purified factor XIIa and ATIII, we found that heparin (0.7 to 34.0 U/mL) increased the rate of inhibition of factor XIIa.

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However, at heparin concentrations usually achieved during anticoagulant therapy (0.7 U/mL), the inhibition was accelerated only four-fold. This implies only a 6% contribution to the inhibitory effect of plasma. This suggestion was confirmed by the observation that heparin (1.5 U/mL) added to factor XIIa did not produce a detectable enhancement of the rate of inhibition of factor XIIa. Furthermore, using purified factor XIIf and antithrombin III, heparin (3.6 to 57.2 U/mL) increased the inactivation rate constant of factor XIIf by 1.6 to 14.0 times. This small effect was confirmed by the observation that heparin at a concentration greater than that sufficient for anticoagulation (1.4 U/mL) did not modify the inactivation rate of factor XIIf by prekallikrein-deficient plasma, and thus C1 inhibitor remains the major inhibitor even in the presence of heparin. From this study and our previous investigations on the effect of heparin on the inhibition of kallikrein and factor XIa, we conclude that heparin does not significantly affect the protease activity of purified contact activation factors or the activities expressed by these proteases in plasma.

Pixley, R. A., Schapira, M., and *Colman, R. W.*

Blood 66:198-203, 1985.

Other support: American Heart Association, Southeastern Pennsylvania Chapter; National Institutes of Health Individual National Research Service Award; and Swiss National Science Foundation.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia, and Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

THE REGULATION OF HUMAN FACTOR XIIa BY PLASMA PROTEINASE INHIBITORS

Studies of the inactivation of factor XIIa by plasma protease inhibitors in purified systems and in plasma were initiated to determine the relative importance of these inhibitors to the neutralization of factor XIIa. Factor XIIa was measured by the amidolysis of H-D-prolyl-L-phenylalanyl-L-arginine-p-nitroanilide dihydrochloride or by coagulant activity. C1 inhibitor (C1INH), α_2-antiplasmin (α_2AP), α_2-macroglobulin (α_2M), and antithrombin III (ATIII) inhibited factor XIIa with second-order rate constants of 2.2×10^5, 1.1×10^4, 5.0×10^3, and 1.3×10^3 $M^{-1}min^{-1}$. Factor XIIa activity was not affected by α_1-proteinase inhibitor. Incubation of ^{125}I-radiolabeled factor XIIa resulted in 1:1 stoichiometric complexes with C1INH (M_r 100,000), ATIII (M_r 125,000), and α_2AP (M_r 150,000 and 125,000) using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Incubation of ^{125}I-Factor XIIa with α_2M resulted in a component of M_r 85,000 on a reduced sodium dodecyl sulfate-polyacrylamide gel, indicating that a subunit of factor XIIa was covalently bound to a proteolyzed portion of α_2M. The relative effectiveness of each inhibitor at plasma concentrations was 61:2:3:1 for C1INH, α_2AP, α_2M, and ATIII, respectively. Kinetic studies of the inactivation of purified factor XIIa added to various plasmas containing different concentrations of C1INH verified the predictions from the purified systems. Gel filtration of radiolabeled factor XIIa incubated with plasma confirmed that factor XIIa-C1INH was the major complex. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that the complexes in plasma had the same molecular size as those with purified inhibitors. C1INH functions as the predominant inhibitor of factor XIIa in plasma.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

Pixley, R. A., Schapira, M., and *Colman, R. W.*

The Journal of Biological Chemistry 260(3):1723-1729, 1985.

Other support: National Institutes of Health; American Heart Association, Southeastern Pennsylvania Chapter; and Swiss National Science Foundation.

From the Thrombosis Research Center and the Department of Medicine, Temple University School of Medicine, Philadelphia, and the Division de Rhumatologie, Hopital Cantonal Universitaire, Geneva, Switzerland.

EFFECT OF CLEAVAGE OF THE HEAVY CHAIN OF HUMAN PLASMA KALLIKREIN ON ITS FUNCTIONAL PROPERTIES

Human plasma kallikrein consists of an N-terminal heavy chain of molecular weight (mol wt) 52,000, linked by disulfide bonds to two light chain variants (mol wt 36,000 or 33,000). Although the active catalytic site of kallikrein resides on the C-terminal light chain, the role of the N-terminal heavy chain is less clear. We therefore studied an enzyme designated β-kallikrein, containing a single cleavage in the heavy chain (mol wt 28,000 + 18,000) and compared it to the enzyme, β-kallikrein, with an intact heavy chain. The rates of inactivation by C1 inhibitor of plasma α- and β-kallikreins were kinetically identical, as measured by residual amidolytic activity, after various times of incubation with the inhibitor. Both enzymes reacted completely with C1 inhibitor after 18 hours and formed identical C1 inhibitor-kallikrein complexes of mol wt 195,000. The rate of activation of factor XII by α-kallikrein and β-kallikrein was similar. In contrast, the rate of cleavage of high molecular weight kininogen (HMWK) by α-kallikrein was at least fivefold faster and the ratio of coagulant activity to amidolytic activity was fourfold greater than for β-kallikrein. Plasma α-kallikrein, at concentrations potentially achievable in plasma, induced aggregation of neutrophils, but β-kallikrein failed to elicit this response. In addition, human neutrophils that had been pretreated with cytochalasin B released 2.46 ± 0.10 $\mu g/10^7$ cells of elastase antigen, but β-kallikrein released only 0.25 ± 0.10 $\mu g/10^7$ cells. These observations suggest that cleavage of the heavy chain influences the rate of cleavage of HMWK and decreases its coagulant activity. Moreover, an intact heavy chain appears to be requisite to support the ability of kallikrein to aggregate neutrophils and release elastase.

Colman, R. W. et al.

Blood 65(2):311-318, 1985.

Other support: National Institutes of Health and the Swiss National Science Foundation.

[illegible] the Thrombosis Research Center and the Hematology-Oncology Section, Department of Medicine, Temple University School of Medicine, Philadelphia, and the Graduate Hospital Research Center, Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

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REGULATION OF THE COAGULANT ACTIVITY AND SURFACE BINDING OF HIGH MOLECULAR WEIGHT KININOGEN

Events in the formation of the enzymes in the intrinsic coagulation pathways can be conceptualized as three reaction clusters. The procofactors of the two later steps, factors VIII and V, are each proteolyzed by thrombin to active cofactors, factors VIIIa and Va, which are then bound to phospholipid micelles or platelet membranes. Each cofactor then serves as a "receptor" to which the enzymes, factors IXa and Xa, bind prior to activating the substrates, factor X and prothrombin. The cofactors, factors VIIIa and Va, are then inactivated by another enzyme protein, activated protein C. Our recent studies indicate that the procofactor of the contact system is similarly subject to activation by kallikrein to an active cofactor prior to binding to an activating surface.

The activation of the contact system is probably initiated by the binding of plasma factor XII to a negatively charged surface where autoactivation occurs for form factor XIIa. The substrates of factor XIIa, prekallikrein (PK) and factor XI, exist in bimolecular complexes with the contact system procofactor, high molecular weight kininogen (HMWK), which is required to transport them to an activating surface. Here, on the surface, these zymogens are converted to the active enzymes, kallikrein (K) and factor XIa, by activated factor XII. We have recently delineated the mechanism by which the binding of XII and HMWK on the surface is coordinated. XIIa first cleaves PK, which in turn converts the procofactor, HMWK, to an active cofactor, HMWKa, with the ability to bind to an activating surface and express coagulant activity by bringing PK and XI into proper apposition for cleavage by XIIa. XIIa then cleaves XI to XIa.

Colman, R. W., Silver, L. D., Purdon, A. D., Chang J. J., and Scott, C.F.

Transactions of the Association of American Physicians XCVII:113-123, 1984.

Other support: National Institutes of Health and Specialized Center of Research on Thrombosis.

From the Thrombosis Research Center and the Hematology-Oncology Section, Department of Medicine, Temple University School of Medicine, Philadelphia.

PLASMA PREKALLIKREIN ASSAY: REVERSIBLE INHIBITION OF C1 INHIBITOR BY CHLOROFORM AND ITS USE IN MEASURING PREKALLIKREIN IN DIFFERENT MAMMALIAN SPECIES

The assay of plasma prekallikrein requires activation of prekallikrein to kallikrein and sufficient inactivation of the plasma protease inhibitors of kallikrein to accurately measure the generated kallikrein activity. One method of elimination of the plasma protease inhibitors to kallikrein is to chemically pretreat the plasma. Methylamine has previously been employed to selectively inactivate α_2-macroglobulin. Our study examines the effect of sequential preincubation of plasma with chloroform and methylamine on the plasma prekallikrein assay. Chloroform was demonstrated to be a chemical inhibitor of purified C1 inhibitor, but α_2-macroglobulin was not. Chloroform inhibition of C1 inhibitor was not caused by precipitation of the protein into the interface between the water and organic solvent phase. Greater than 95% of C1 inhibitor antigen was recovered in the supernatant of chloroform-treated purified C1 inhibitor,

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and chloroform-saturated buffer inhibited purified C1 inhibitor. Chloroform did not dissociate a preformed complex of kallikrein and C1 inhibitor, but its inhibition of C1 inhibitor was reversible. The addition of methylamine to plasma pretreated with chloroform in the plasma prekallikrein assay allowed for only a slight increase in the amount of kallikrein measured a 1 minute kaolin activation times, but provided for sustained measurement of activated prekallikrein when kaolin activation times were 5 to 7 minutes. Without chemical pretreatment, prekallikrein was not measurable in rabbit plasma. Both rabbit and pig plasma prekallikrein were measurable after exposure of the plasma to chloroform and methylamine, although the peak activation times and the contribution of each animal's protease inhibitors varied with the species. Our results show that chloroform is a reversible inhibitor of C1 inhibitor, and that the plasma prekallikrein assay in which it is used is useful for the measurement of prekallikrein in nonhuman mammalian plasma samples.

Schmaier, A. H., Gustafson, E., Idell, S., and *Colman, R. W.*

The Journal of Laboratory and Clinical Medicine 104(6):882-892, 1984.

Other support: National Heart, Lung and Blood Institute, American Heart Association, and National Institutes of Health.

From the Thrombosis Research Center, Hematology/Oncology and Pulmonary Sections, Department of Medicine, Temple University School of Medicine, Philadelphia.

THE EFFECT OF HIGH MOLECULAR WEIGHT KININOGEN ON SURFACE-ADSORBED FIBRINOGEN

High molecular weight kininogen (HMWK) plays an important role in altering the association of plasma fibrinogen with surfaces. Plasma initially deposits fibrinogen onto most materials, but on hydrophilic surfaces within 10 min adsorbed plasma fibrinogen cannot be detected on the surface by anti-fibrinogen antisera. However, using HMWK-deficient plasma, fibrinogen remains immunologically identifiable. The inter-relationship of adsorbed plasma fibrinogen with kininogen on hydrophilic surfaces is studied further using glass slides stained for protein with Coomassie Blue, and oxidized silicon crystal slices in an automated ellipsometer. On glass slides when plasma that is deficient in both low molecular weight kininogen (LMWK) and HMWK is reconstituted with HMWK (0.40 Units/ml), fibrinogen is no longer detected on the surface. This finding is specific for HMWK, since, when the same plasma is reconstituted with LMWK (220 μg/ml), the amount of fibrinogen detected on the surface is unchanged. The alteration of surface-adsorbed fibrinogen by HMWK is not due to plasmin-induced fibrinolysis, since it occurs in plasminogen-free plasma. In the ellipsometer, surface adsorption of normal plasma is associated with a significantly less ($p < 0.0005$) thick protein layer (1.99 $\pm$ 0.09 degree change in azimuth) than plasmas deficient in HMWK (2.32 $\pm$ 0.11). Using ellipsometry, HMWK in plasma is shown to shorten the time in which immunologically detectable surface-adsorbed fibrinogen was removed or altered. These studies in a whole plasma system present further evidence that HMWK specifically modifies the association of plasma fibrinogen with hydrophilic surfaces.

Schmaier, A. H., Silver, L., Adams, A. L., Fischer, G. C., Munoz, P. C. Vroman, L., and *Colman, R. W.*

Thrombosis Research 33:51-67, 1984.

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Other support: National Heart, Lung and Blood Institute, American Heart Association, a Temple University Biomedical Research Support Grant, and National Institutes of Health.

From the Hematology/Oncology Section and Thrombosis Research Center, Temple University School of Medicine, Philadelphia; and the Interface Laboratory, Veterans Administration Medical Center, Brookly, New York.

CHROMOSOMAL TRANSFER IN MOUSE ERYTHROLEUKEMIA CELLS SHOWS THAT TRANS REGULATION MEDIATES TISSUE SPECIFIC AND DEVELOPMENTALLY SPECIFIC PATTERNS OF GLOBIN GENE EXPRESSION

The data presented in this paper have suggested that the activation of embryonic globin genes in the K562 cell line arose through a single mutation which led to altered expression of a diffusible regulatory factor capable of activating embryonic globin genes. To test the hypothesis that embryonic globin gene activation had occurred in the K562 cell through altered expression of a trans regulatory factor, rather than a mutation in cis to the gene, it was decided to introduce the transcriptionally active zeta globin genes of the K562 cell into the tetraploid Friend mouse erythroleukemia cell in which adult but not embryonic globin gene expression occurs. In this study the authors also used restriction fragments length polymorphisms to show that both homologues of chromosome 16 which are present in the K562 cells contain alpha and zeta globin genes which behave in an identical manner when transferred to the mouse erythroleukemia cells. The results show that the mutation which led to the activation of human embryonic globin genes in the K562 cells is asyntenic to the zeta globin genes. These results also suggest that the K562 cell contains a trans regulatory factor which is competent to support the expression of the human embryonic globin genes.

Deisseroth, A. B.

In: Stamatoyannopoulof, G. and Nienhuis, A. (eds.): *Experimental Approaches for the Study of Hemoglobin Switching*. New York: Alan R. Liss, 1982, 293-303.

Other support: Veterans Administration and National Heart, Lung and Blood Institute.

From the Department of Medicine, University of California at San Francisco, Hematology/Oncology Unit, and the Veterans Administration Medical Center, San Francisco.

PLASMA PROTEIN AND LIVER mRNA LEVELS OF TWO CLOSELY RELATED MURINE α1-PROTEASE INHIBITORS DURING THE ACUTE PHASE REACTION

Plasma levels of α1-PI(T) and α1-PI(E), two closely related murine alpha 1-protease inhibitors, having affinities for trypsin and elastase, respectively, were compared to changes in specific liver mRNA levels after induction of the acute-phase reaction by subcutaneous injection of turpentine. In earlier, qualitative experiments, an increase in plasma levels of α1-PI(E) but not α1-PI(T) during the acute-phase reaction had been shown. It is now shown that stimulation of plasma α1-PI(E) levels reaches a maximum of 35-50% above baseline 12h after induction of the acute-phase response using either a functional or immunological assay to measure protease inhibitor activity. Consistent with earlier observations, little or no change in plasma levels of α1-PI(T) is

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seen. Determination of mRNA levels in the mouse liver specific for α1PI(E) and α1-PI(T) was accomplished using a cell-free translation system followed by immunoprecipitation of the ^{35}S-labeled protease inhibitors. The apparent molecular weights of α1-PI(E) and α1-PI(T) synthesized *in vitro* are 42K and 46K, respectively. Apparent molecular weights of the native proteins in plasma are 55K and 65K. Unexpectedly, mRNA levels for both α1-PI(E) and α1-PI(T) were found to increase after induction of the acute-phase reaction. Maximal stimulation for both mRNAs was approximately 300% and occurred 9h after turpentine administration. Under these conditions, levels of translatable albumin mRNA in the mouse liver decreased to 40% of baseline in 6-9h.

Frazer, J. M., Nathoo, S. A., Katz, J., Genetta, T. L., and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 39(1):112-119, 1985.

Other support: National Science Foundation.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

REGULATION OF PLASMA FACTOR XIII BINDING *IN VITRO*

The binding of plasma factor XIII to fibrinogen or fibrin that has been chemically or enzymatically induced to polymerize was studied. Factor XIII binding was assayed using a ^{3}H-putrescine incorporation assay and an ^{125}I-plasma factor XIII binding assay. More than 80% of the native and radiolabeled plasma factor XIII was bound to fibrin I formed by reptilase in EDTA, citrate, or heparin anticoagulated plasma. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{125}I-plasma factor XIII bound to fibrin I or fibrin II formed by reptilase or thrombin in the presence of EDTA demonstrated the b_2-subunit remained bound to the a-chains or thrombin-cleaved a-chains. In the presence of calcium chloride and thrombin, the b_2-subunit dissociated and factor XIIIa was bound. Protamine sulfate caused fibrinogen polymerization in the absence of divalent cations and reduced both plasma factor XIII and immunologic fibrinogen levels. Fibrinogen polymerized by protamine sulfate bound plasma factor XIII and the a_2-subunit of ^{125}I-platelet factor XIII. Plasma factor XIII was also bound to sonicated non-cross-linked fibrin II in either normal plasma or afibrinogenemic plasma. Plasma levels of several coagulation proteins were unchanged after the addition of reptilase, protamine sulfate or sonicated fibrin to plasma. These results demonstrate that a specific binding site for the a_2-subunit of plasma factor XIII is present on polymerized fibrinogen, fibrin I and fibrin II. Furthermore, the presence of divalent cations, thrombin-cleavage of plasma factor XIII and release of fibrinopeptides A or B are not required for plasma factor XIII binding to polymerized fibrinogen and fibrin.

Greenberg, C. S., Dobson, J. V., and Miraglia, C. C.

Blood 66(5):1028-1034, 1985.

Other support: American Heart Association.

From the Departments of Medicine and Pathology, Duke University Medical Center, Durham, NC.

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MEASUREMENT OF BLOOD COAGULATION FACTOR XIIIa FORMATION IN PLASMA CONTAINING GLYCYL-L-PROLYL-L-ARGINYL-L-PROLINE

A method is described to directly measure the formation of blood coagulation Factor XIIIa in platelet-poor plasma unmodified by heat. The synthetic peptide glycyl-L-prolyl-L-arginyl-L-proline, a fibrin-polymerization inhibitor, was used to prevent clotting of platelet-poor plasma. Plasma was diluted to a final concentration of 2.5% (v/v) in 0.1 M Tris-HCl, pH 8.5, buffer containing 25% glycerol, 5 mM calcium chloride, and 0.25 mM glycl-L-prolyl-L-arginyl-L-proline and then activated by thrombin (20 U/ml) for 15 min. The Factor XIIIa-catalyzed incorporation [^{3}H]putrescine into Hammersten casein was used to measure Factor XIIIa formation. The assay detected Factor XIIIa in 2.5 to 50 μl of thrombin-treated plasma. When purified Factor XIII was added to Factor XIII-deficient plasma, there was complete recovery of the Factor XIII added. Glycl-L-prolyl-L-arginyl-L-proline did not inhibit Factor XIIIa activity in thrombin-treated plasma or purified platelet Factor XIIIa. Glycerol stabilized Factor XIIIa activity in thrombin-treated plasma and buffer for 60 min. The presence of fibrinogen in plasma did not modify the assay results. The time course of thrombin-catalyzed Factor XIIIa formation in platelet-poor plasma containing glycyl-L-prolyl-L-arginyl-L-proline was directly measured using the assay.

Miraglia, C. C. and *Greenberg, C. S.*

Analytical Biochemistry 144:165-171, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, Duke University Medical Center, Durham, NC.

THE EFFECT OF FIBRIN POLYMERS ON THROMBIN-CATALYZED PLASMA FACTOR XIIIa FORMATION

The effect of fibrin polymers on thrombin-catalyzed factor XIIIa formation was studied in afibrinogenemic plasma. Fibrin polymers derived from des A fibrinogen and des A,B fibrinogen increased six-fold the rate of thrombin-catalyzed factor XIIIa formation in the presence of EDTA. Calcium chloride accelerated factor XIIIa formation 14-fold in the presence of des A,B fibrinogen without increasing the rate of thrombin formation. Fibrinopeptides A and B had no effect on factor XIIIa formation in afibrinogenemic plasma. Des A,B fibrinogen reduced by 20- to 40-fold the thrombin concentration required to activate factor XIII. Glycyl-L-prolyl-L-arginyl-L-proline (gly-pro-arg-pro), a fibrin polymerization inhibitor, inhibited des A and des A,B fibrinogen from enhancing thrombin-catalyzed factor XIIIa formation. Gly-pro-arg-pro did not modify factor XIIIa formation in afibrinogenemic plasma and did not inhibit thrombin cleavage of the chromogenic substrate S-2238. These results demonstrate that fibrin polymers accelerate thrombin-catalyzed plasma factor XIIIa formation.

Greenberg, C. S. and Miraglia, C. C.

Blood 66(2):466-469,1985.

Other support: National Institutes of Health and American Heart Association.

From the Departments of Medicine and Pathology, Duke University, Durham, NC.

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RADIOIMMUNOASSAY FOR NEUROPEPTIDE Y (NPY): CHROMATOGRAPHIC CHARACTERIZATION OF IMMUNOREACTIVITY IN PLASMA AND TISSUE EXTRACTS

A sensitive and specific radioimmunoassay was developed to determine the occurrence and concentration of neuropeptide Y (NPY) in plasma and tissue extracts. Furthermore, NPY-like immunoreactivity (NPY-Li) was characterized by means of three different chromatographic systems. The NPY antiserum used (NI) did not cross-react with related peptides of the pancreatic polypeptide family except avian pancreatic polypeptide (1% cross-reactivity). Unextracted plasma contained high molecular weight proteins which interfered in the assay. Acid ethanol extraction removed this protein interference allowing a 90% recovery of NPY-Li. The content of NPY-Li in human plasma from healthy subjects was close to or below the detection limit (< 22 pmol/l). Sympathetic nerve stimulation in the cat increased the output of NPY-Li from the splenic vein suggesting the release of this peptide upon sympathetic activation. The major peak of NPY-Li in spleen extracts and splenic vein plasma co-eluted with synthetic porcine NPY and a minor peak with larger Stokes radius was also present. the present radioimmunoassay enables further studies on the physiological and pathophysiological role of NPY.

Theodorsson-Norheim, E., Hemsen, A. and *Lundberg, J. M.*

Scandinavian Journal of Clinical & Laboratory Investigation 45:355-365, 1985.

Other support: The Swedish Medical Research Council; the Karolinska Institute and the Astra Foundation.

From the Department of Clinical Chemistry, Karolinska Hospital, and the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

IDENTIFICATION OF HUMAN PLATELET MEMBRANE FIBRINOGEN RECEPTORS BY IMMUNOCHEMICAL TECHNIQUES

The fibrinogen receptors of platelets were investigated with the use of three types of anti-platelet membrane antibodies and three types of platelets. We found that antisera raised in rabbits against membranes prepared from human intact, chymotrypsin- or pronase-treated platelets inhibited the fibrinogen-induced aggregations of ADP-stimulated intact platelets, chymotrypsin-treated platelets and pronase-treated platelets. These antisera also blocked the binding of ^{125}I-fibrinogen to ADP-stimulated intact, chymotrypsin-treated, and pronase-treated platelets. These results suggest that all three antisera blocked the interaction of fibrinogen with its receptor on the surface of the three types of platelets studied. Fibrin clot retraction by intact platelets was also inhibited by these three antibodies indicating an important role of platelet membrane proteins in clot retraction. As demonstrated by techniques using ^{125}I-surface labeling, *Staphylococcus aureus* immunoprecipitation, SDS-polyacrylamide gel electrophoresis and autoradiography, anti-intact platelet membrane antibody immunoprecipitated the membrane glycoproteins GPIIb, GPIII and a protein with an apparent molecular weight of 66,000 from detergent solubilized surface ^{125}I-iodinated chymotrypsin-treated platelets. Anti-chymotrypsin and anti-pronase-treated platelet membrane antisera immunoprecipitated mostly GPIII and the 66,000 molecular weight protein from detergent solubilized, surface ^{125}I-iodinated chymotrypsin-treated platelets.

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The 66,000 Mr protein was not found on the surface of intact (unstimulated) platelets which do not bind ^{125}I-fibrinogen and are not aggregated by fibrinogen without the prior addition of ADP. The ability of anti-platelet membrane antibodies to block fibrinogen-induced platelet aggregation and fibrinogen binding to platelets correlated with their ability to immunoprecipitate a 66,000 Mr protein from the platelet surface. It is proposed that the 66,000 Mr protein may be the fibrinogen binding domain of GPIII which becomes permanently exposed on the surface of chymotrypsin and pronase-treated platelets following proteolysis and which becomes exposed upon stimulation of intact platelets by agents such as ADP.

Kornecki, E., Tuszynski, G. P., and *Niewiarowski, S.*

Hematologia 17(3):387-398, 1984.

Other support: National Institutes of Health and National Research Service.

From the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

MEMBRANE FLUIDITY AND PLATELET FIBRINOGEN RECEPTOR EXPOSURE BY PROTEOLYTIC ENZYMES

Incubation of platelets with pronase or chymotrypsin results in the exposure of fibrinogen receptors. We determined that these enzymes did not affect the membrane fluidity as evaluated by the depolarization of the fluorescence of 1,6-diphenyl-1,3,5 hexatriene (DPH). There was no significant difference in either the depolarization or in its temperature dependence for control, pronase or chymotrypsin-treated platelets. Thus, it can be concluded that the exposure of fibrinogen receptors on the platelet surface by proteolytic enzymes does not depend on the changes of membrane fluidity. We also propose that the proteolytic enzymes do not cause a major alteration in the extent of protein chains embedded in the lipid layers of the platelet membranes.

Simons, E., Whitin, J. C., Morinelli, T. A., Stewart, G. J., and *Niewiarowski, S.*

Thrombosis Research 39:91-96, 1985.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Department of Physiology, Temple University Health Sciences Center, Philadelphia, and the Department of Biochemistry, Boston University School of Medicine, Boston.

AGGREGATION OF CHYMOTRYPSIN-TREATED THROMBASTHENIC PLATELETS IS MEDIATED BY FIBRINOGEN BINDING TO GLYCOPROTEINS IIb AND IIIa

Previous experiments demonstrated that chymotrypsin, but not adenosine diphosphate (ADP), exposed fibrinogen binding sites on platelets from patients with Glanzmann's thrombasthenia. Three of these patients have been reexamined, and previous observations were confirmed. The quantity of iodine 125-labeled glycoprotein IIb (GPIIb) and glycoprotein IIIa (GPIIIa) on the platelets of these patients was

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considerably less than normal but was detectable by immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography. The amount of residual GPIIb and GPIIIa as measured by binding studies with radiolabeled monoclonal antibodies was between 3% and 12% of the normal value. Platelet suspensions from these patients did not aggregate with fibrinogen and did not bind ^{125}I-fibrinogen on stimulation with ADP. However, incubation of these platelets with chymotrypsin or pronase resulted in fibrinogen binding and platelet aggregation. Monocolonal antibodies specific for the GPIIb-GPIIIa complex blocked both the fibrinogen binding and the aggregation of enzyme-treated platelets. The treatment of washed platelets of a fourth thrombasthenic patient with ADP or with chymotrypsin failed to result in fibrinogen binding and aggregation. However, the level of GPIIb and GPIIIa on these platelets as measured by a Western blot technique and by monoclonal antibody binding amounted to < 0.35% to 0.5% of normal values. In conclusion, fibrinogen binding sites exposed on thrombasthenic platelets by chymotrypsin are derived from GPIIb-GPIIIa molecules. Aggregation of chymotrypsin-treated thrombasthenic platelets by fibrinogen appears to represent a sensitive test for detection of functionally active GPIIb-GPIIIa complex on the platelet surface.

Niewiarowski, S. et al.

Journal of Laboratory and Clinical Medicine 106(6):651-660, 1985.

Other support: National Institutes of Health and the American Heart Association.

From the Thrombosis Research Center and Department of Physiology, Temple University Health Sciences Center, Philadelphia; the Hematology/Oncology Section, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia; and the Hâpital Lariboisière, Paris, France.

IDENTIFICATION OF PI^{AI} ALLOANTIGEN DOMAIN ON A 66 kDa PROTEIN DERIVED FROM GLYCOPROTEIN IIIa OF HUMAN PLATELETS

Incubation of platelets with chmyotrypsin leads to the exposure of fibrinogen receptors and to the appearance of a 66 kDa membrane component on the surface of platelets. Both glycoprotein IIIa (GP IIIa) and a 66 kDa component were precipitated from detergent extracts of solubilized, surface radiolabeled chymotrypsin-treated platelets by human anti-PI^{AI} antisera. Moreover, the presence of the PI^{AI} antigen was identified on GP IIIa (but not on GP IIb) and on a 66 kDa protein by means of immunoblot procedures using platelet Triton X-114 extracts and these purified proteins. Anti-PI^{AI} antiserum did not recognize GP IIIa on the surface of intact (untreated) platelets nor the 66 kDa protein on the surface of chymotrypsin-treated platelets of PI^{AI}-negative individuals. The present data demonstrate directly that the 66 kDa protein is derived from GP IIIa and contains the PI^{AI} alloantigen.

Kornecki, E., Chung, S.-Y, Holt, J. C., Cierniewski, C. S., Tuszynski, G. P., and *Niewiarowski, S.*

Biochemica et Biophysica Acta 818:285-290, 1985.

Other support: National Institutes of Health and the National Heart, Lung and Blood Institute.

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From the Thrombosis Research Center, Temple University Health Science Center, Philadelphia, and the Department of Psychiatry, Neurosciences Research Unit, University of Vermont, Burlington.

THE INTERACTION OF HUMAN PLATELET THROMBOSPONDIN WITH FIBRINOGEN: THROMBOSPONDIN PURIFICATION AND SPECIFICITY OF INTERACTION.

Human platelet thrombospondin (TSP) was purified to homogeneity by chromatography on fibrinogen coupled to cyanogen bromide-activated Sepharose. The yield of TSP was 1.3 mg or approximately 22% of that present in platelet-rich plasma as determined by radioimmunoassay. It analyzed on discontinuous sodium dodecyl sulphate gels as a single band having apparent molecular weights of 180,000 and ≥ 400,000 under reducing and nonreducing conditions, respectively. Amino acid analysis gave results similar to previously published values. Antibodies raised in rabbits were monospecific as evaluated by radioimmunoassay. In double immunodiffusion tests, these antibodies gave one line of identity against TSP purified by this procedure and TSP purified by published procedures, confirming the identity of the material isolated. The protein possesses no lectin-like activity.

The specificity of the TSP-fibrinogen interaction was investigated. TSP binding to fibrinogen-Sepharose occurred in the presence of EDTA, indicating that calcium and magnesium ions are not required for interaction of TSP with fibrinogen. The binding of TSP to fibrinogen-Sepharose was quantitatively blocked by pretreatment with an antibody to the cyanogen bromide cleavage fragment composed of residues 241-476 of the carboxyl-terminal end of the α chain of fibrinogen. Antibodies against the D and E domains of fibrinogen had no effect on the binding. Excess fibrinogen (30 mg/ml) added to platelet extract quantitatively inhibited binding of TSP to fibrinogen-Sepharose. TSP preferentially bound to uncross-linked fibrin, suggesting that the TSP-fibrinogen binding site is unavailable in cross-linked fibrin. These results indicate that TSP binds specifically to immobilized fibrinogen or uncross-linked fibrin through determinants present in the carboxyl-terminal portion of the α chain and that these interactions do not require calcium or magnesium ions.

Tuszynski, G. P., Srivastava, S., Switalska, H. I., Holt, J. C., Cierniewski, C. S., and *Niewiarowski, S.*

The Journal of Biological Chemistry 260(2):12240-12245, 1985.

Other support: U. S. Department of Health and Human Services and the American Heart Association, Pennsylvania Keystone Affiliate.

From the Thrombosis Research Center and the Department of Physiology, Temple University School of Medicine, Philadelphia.

LOSS OF FIBRINOGEN RECEPTORS FROM THE PLATELET SURFACE DURING SIMULATED EXTRACORPOREAL CIRCULATION

In vitro recirculation of fresh human heparinized blood in an extracorporeal circuit with a membrane oxygenator decreased fibrinogen-induced platelet aggregation and diminished the number of fibrinogen receptors and glycoprotein IIb/IIIa (GPIIb/GPIIIa) antigenic sites on the platelet surface. In seven experiments, the mean ± SD K_m value for fibrinogen (i.e., molar concentration of fibrinogen required to cause 50% of the maximal rate of aggregation) was 1.58×10^{-7} mol/L ± 0.68×10^{-7} mol/L. After recirculation, this value increased to 3.8×10^{-7} mol/L ± 1.94×10^{-7} mol/L ($P \leq 0.025$).

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The maximal aggregation rate of chymotrypsin-treated platelets decreased by 40% after 2 hours of recirculation ($P \leq 0.025$). The number of fibrinogen receptors on platelets, which were treated with chymotrypsin after recirculation, decreased from 41,370 ± 24,000 to 13,230 ± 10,230/platelet under the same conditions ($P \leq 0.025$). The number of antigenic sites for monoclonal antibody reacting with GPIIb/GPIIIa complex of adenosine diphosphate-stimulated platelets decreased from 34,200 ± 5,940 to 19,500 ± 9,680/platelet after recirculation ($P \leq 0.025$). Prostaglandin E_1 (0.3 μmol/L) in the perfusion circuit preserved the ability of platelets to react with fibrinogen. In conclusion, the loss of fibrinogen receptors from the surface of platelet membranes results from the interaction of platelets with the surfaces of perfusion circuits.

Musial, J., *Niewiarowski, S.*, Hershock, D., Morinelli, T. A., Colman, R. W., and Edmunds, L. H. Jr.

Journal of Laboratory and Clinical Medicine 105:514-522, 1985,

Other support: National Institutes of Health.

From the Department of Cardiovascular Surgery, University of Pennsylvania Hospital and the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

RADIOIMMUNOASSAY OF HUMAN PLATELET THROMBOSPONDIN: DIFFERENT PATTERNS OF THROMBOSPONDIN AND β-THROMBOGLOBULIN ANTIGEN SECRETION AND CLEARANCE FROM THE CIRCULATION

A method for radioimmunoassay of human thrombospondin was developed. Monospecific precipitating anti-human thrombospondin antibody was raised in rabbits after injection of thrombospondin purified by fibrinogen-agarose chromatography and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The linear portion of the thrombospondin radioimmunoassay standard curve was 0.5 to 20 ng/ml. Normal platelets and platelet-poor plasma contained 28,900 ± 14,500 ng thrombospondin per 10^9 platelets and 60.6 ± 10.7 ng/ml (mean ± SD), respectively. Using radioimmunoassays for β-thromboglobulin and thrombospondin antigens, we compared platelet location and secretion of these proteins. Both antigens shared similar distributions in platelet subcellular fractions with the largest amount localized to platelet α-granules. With thrombin (0.25 U/ml) as a platelet agonist, 62.4% and 19.5% of total β-thromboglobulin and thrombospondin, respectively, were secreted from suspensions of washed human platelets. Because only 20% of the total platelet thrombospondin was secreted, further studies were initiated to determine whether the remaining thrombospondin became localized on the activated platelet membrane. ^{125}I-Fab antithrombospondin specifically bound to activated platelets but not to unstimulated platelets. In contrast, ^{125}I-Fab anti-β-thromboglobulin did not bind to activated platelets. Plasma clearance of human β-thromboglobulin (half-life fast 7.6 minutes, slow 56.6 minutes) and of human thrombospondin (half-life fast 29.9 minutes, slow 190 minutes) followed a biphasic exponential curve. In conclusion, both β-thromboglobulin and thrombospondin are located in platelet α-granules, but they show a different pattern of secretion and expression on the platelet membrane and plasma clearance.

Switalska, H. I., *Niewiarowski, S. et al.*

Journal of Laboratory and Clinical Medicine 106:690-700, 1985.

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Other support: National Institutes of Health and the American Heart Association.

From the Thrombosis Research Center, Departments of Physiology and Medicine, Temple University Health Sciences Center, Philadelphia.

INTERACTION OF CHRONIC CIGARETTE AND ETHANOL USE ON MYOCARDIUM

Chronic cigarette use is common in persons who habitually use other cardioactive agents that have been causally associated with heart disease. This study was undertaken to determine if cigarette use intensifies the abnormalities of myocardial function and composition observed in experimental alcoholism over an 18-month period. Young adult male beagles with tracheostomy were divided into four groups. There were 10 controls (group 1); 9 smoked seven cigarettes per day (group 2); 7 were fed ethanol as 20% of calories (group 3), and 6 received both ethanol and cigarettes (group 4). After a period of 18 months, left ventricular function was assessed under anesthesia. Heart rate, left ventricular end-diastolic pressures and volumes (indicator dilution) did not differ in the four groups. An index of contractility derived by normalizing peak dP/dt for pre- and after-load was reduced significantly below the level of 2.4 ± 0.7 cm/s in control dogs to 1.41 ± 0.35 in group 2, 1.1 ± 0.38 in group 3, and 1.28 ± 0.17 in the ethanol cigarette group (each $p \leq 0.002$). Arterial pressures were moderately elevated above group 1 in all three experimental groups without evidence of left ventricular hypertrophy. In contrast to smoking, which elicited no abnormalities of myocardial cation composition, ethanol reduced myocardial potassium and sodium in group 3 without a gain of water content. In group 4, no further decline of tissue cations was observed. Thus, cigarette use when combined with ethanol over a relatively long period, produced no greater myocardial abnormalities than ethanol alone and may not be essential to the genesis of cardiomyopathy in alcoholics.

Ahmed, S. S., Torres, R., and *Regan, T. J.*,

Clinical Cardiology 8(3):129-136, 1985.

From the Division of Cardiovascular Diseases, Department of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark.

ISOLATION AND CULTURE OF PULMONARY ENDOTHELIAL CELLS

Methods for isolation, identification and culture of pulmonary endothelial cells are now routine. In the past, methods of isolation have used proteolytic enzymes to detach cells; thereafter, traditional methods for cell passaging have used trypsin/EDTA mixtures. Cells isolated and passaged using proteolytic enzymes have been useful in establishing the field and in verifying certain endothelial properties. However, there is a growing awareness of the role of endothelial cells in processing vasoactive substances, in responding to hormones and other agonists, and in cell-cell interactions with other cell types of the vascular wall, with blood cells and with cellular products. Consequently, a new requirement has arisen for cells *in vitro* that maintain the differentiated properties of their counterparts *in vivo*. The deleterious effects of trypsin and other proteolytic enzymes commonly used in cell culture on surface structures of endothelial cells such as enzymes, receptors and junctional proteins, as well as on extracellular layers such as the glycocalyx or "endothelial fuzz," have led to the development of methods that avoid use of proteolytic enzymes at both the isolation step and during subsequent subculture. This

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chapter describes traditional methods for isolating pulmonary endothelial cells but emphasizes newer approaches using mechanical harvest and scale-up using microcarriers. The new methods allow maintenance of long-term, large-scale cultures of cells that retain the full complement of surface properties and that maintain the cobblestone monolayer morphology and differentiated functional properties. Methods for identification of isolated cells are therefore also considered as methods for validation of cultures during their *in vitro* lifespan.

Ryan, U. S.
Environmental Health Perspectives 56:103-114, 1984.

Other support: National Institutes of Health and the John A. Hartford Foundation.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

VARICOSE VEINS AS A SOURCE OF ADULT HUMAN ENDOTHELIAL CELLS

Endothelial cells can be harvested from segments of adult human saphenous vein in a varicose condition removed from patients having single or bilateral vein ligation and stripping. The cells are harvested by scraping with a scalpel, seeded on to gelatin coated or Primaria flasks and are passaged by removal with a rubber policeman. The cells cultured in this manner are maintained in a growth medium that is not supplemented with growth factors. The cells grow with a cobblestone monolayer morphology, possess angiotensin converting enzyme activity and react with antibodies to Factor VIII antigen. The cells fluoresce brightly after reaction with monoclonal antibodies specific for human endothelial cells. Thus, stripped varicose vein segments provide a readily available source of endothelial cells.

Ryan, U. S. and White, L. A.

Tissue & Cell 17(2):171-176, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

A POSSIBLE REGULATORY ROLE OF SQUALENE EPOXIDASE IN CHINESE HAMSTER OVARY CELLS

Growth of Chinese hamster ovary cells (CHO) in the presence of 20% lipid depleted serum (LDS) for only 2hr results in an increase in the synthesis of [^{14}C] sterols from [^{14}C] mevalonate and from [^{14}C] squalene compared with cells grown under normal growth conditions in the presence of 10% fetal calf serum (FCS). This enhanced sterol synthesis increases with time of exposure of the cells to LDS. However, exposing these cells for time periods up to 42.52hr to a growth medium containing 20% LDS did not result in enhanced [^{14}C] sterol synthesis from [^{14}C] 2,3,-oxidosqualene. Incubation of these cells with [^{14}C] mevalonate resulted in the accumulation of [^{14}C] squalene regardless of the presence of either LDS or FCS. These results suggest that squalene epoxidase is a regulatory enzyme in the cholesterol biosynthetic pathway in CHO.

Eilenberg, H. and *Shechter, I.*

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

Lipids 19(7):539-543, 1984.

From the Department of Biochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.

UPTAKE OF HIGH-DENSITY LIPOPROTEIN-ASSOCIATED APOPROTEIN A-I AND CHOLESTEROL ESTERS BY 16 TISSUES OF THE RAT *IN VIVO* AND BY ADRENAL CELLS AND HEPATOCYTES *IN VITRO*

The uptake of high-density lipoprotein (HDL)-associated apolipoprotein A-I (apo A-I) and cholesterol esters was estimated in 16 tissues of the rat using rat HDL double labeled with nondegradable tracers; covalently attached ^{125}I-tyramine-cellobiose traced apo A-I, and [^{3}H]cholesteryl linoleyl ether traced cholesteryl esters. Both labels remained associated with the HDL fraction in the plasma, adequately traced their unlabeled counterparts, and were well trapped at their sites of uptake.

Cholesterol ether was taken up at a greater fractional rate than apo-A-I by adrenal, ovary and liver: 7-fold, 4-fold, and 2-fold greater, respectively. The rates of uptake of cholesterol ether and apo-A-I were about equal in the other tissues (except kidney). The disproportionate uptake of HDL cholesterol ether relative to HDL apo A-I was also observed in primary cultures of rat adrenal cells and hepatocytes. Uptake of both moieties in both cell types showed saturability. Both the absolute rate of uptake of [^{3}H]cholesterol ether and the ratio of ether uptake to apo-A-I uptake were greater in adrenal cells than in hepatocytes, consonant with the *in vivo* observations. Very similar results were obtained using HDL biologically labeled with [^{3}]cholesterol *esters*. The disproportionate uptake of [^{3}H]cholesterol ether was not significantly decreased by depletion of apo-E from the HDL, nor by reductive methylation of the apo-E to block its recognition by receptors. However, apo-A-I uptake was decreased, suggesting that apo-E mediates the uptake of particles containing apo-A-I but does not contribute to the disproportionate uptake of [^{3}H]cholesteryl ether.

Glass, C., Pittman, R. C., Civin, M., and *Steinberg, D.*

The Journal of Biological Chemistry 260(2):744-750, 1985.

Other support: National Heart Lung and Blood Institute and National Institute of General Medical Sciences.

From the Department of Medicine, Division of Endocrinology and Metabolism, University of California at San Diego, La Jolla.

ROLE OF THE LOW DENSITY LIPOPROTEIN RECEPTOR IN PENETRATION OF LOW DENSITY LIPOPROTEIN INTO RABBIT AORTIC WALL

The present study was designed to determine whether binding of low density lipoprotein (LDL) to endothelial LDL receptors contributes significantly to the penetration of LDL into normal rabbit aorta. Initial flux rate was used as a measure of uptake of LDL. Reductive methylation of LDL is known to block its recognition by the LDL receptor. Therefore, the difference in flux rates of native LDL and reductively methylated LDL (methyl-LDL) was assumed to represent the receptor-dependent uptake. Native LDL and methyl-LDL were labeled with different isotopes (^{125}I or ^{131}I) and both were injected simultaneously into the same rabbit. After 30 to 60 minutes, trichloracetic acid-precipitable counts were determined in aortic specimens. The initial flux rates, expressed as plasma clearance (nl/g/hr), were 1787 for native LDL and 1924

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for methyl-LDL. The difference was not significant, which suggests that the flux of LDL into the aorta is not significantly dependent upon, or regulated by, endothelial LDL receptors, but is mediated by other mechanisms.

Wiklund, O., Carew, T. E. and *Steinberg, D.*

Arteriosclerosis 5:135-141, 1985.
Other support: National Heart Lung and Blood Institute and the National Institutes of General Medical Sciences.

From the Department of Medicine, University of California at San Diego, La Jolla.

CONTRIBUTION OF ACTIN TO THE STRUCTURE OF THE CYTOPLASMIC MATRIX

The realization that actin is a constituent of nonmuscle cells (1) and the identification of actin filaments in the periphery of such cells (2) opened up a new world of molecular biology. For the first time, it became possible to explain in detail a body of phenomena described over two centuries concerning important aspects of cell shape, movement, and consistency. Although actin comprises only one of several intracellular fiber systems of cells, it is an extremely important one. This essay briefly reviews some present concepts of the contribution of actin to the "cytoplasmic matrix."

Stossel, T. P.

The Journal of Cell Biology 99(1):155-215, Pt.2, July, 1984.

Other support: U. S. Public Health Service, the Muscular Dystrophy Association, and the Edwin S. Webster Foundation.

From the Hematology/Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

NONMUSCLE ACTIN-BINDING PROTEINS

Because the workings of actin-binding proteins depend upon the characteristics of actin itself, the first section of this article summarizes current knowledge of actin assembly. The ensuing review of actin-binding proteins classifies these proteins according to their functions, as has been done previously (Schliwa 1981; Weeds 1982; Craig & Pollard 1983; Stossel 1984). There are actin-binding proteins that bind predominantly to actin monomers and inhibit their self-assembly into filaments. Other proteins bind to one or more actin monomers when they are in the form of filaments, either to the ends of the filaments or in some instances to monomers within the filament, causing the filament to break. They then remain bound to one end of the fractured filament. Still other proteins bind [illegible] to actin monomers in different filaments, which causes the actin filaments to be cross-linked into particular conformations relative to one another. The word "predominant" is very important here because as work progresses on particular actin-binding proteins, their functions tend to become less "pure" and more complex. This review emphasizes biochemical data concerning the structural and functional properties of actin-binding proteins and their interactions with actin *in vitro*. Some effort is made, however, to speculate on how these interactions might operate *in vivo*.

Stossel, T. P. et al.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000

Annual Review of Cell Biology 1:353-402, 1985.

Other support: U. S. Public Health Service and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

COAGULANT ACTIVITIES OF PLATELETS IN CORONARY ARTERY DISEASE

Platelets have been implicated in the pathogenesis of coronary artery disease, and a number of studies have examined platelet function and coagulation parameters in patients with such disease. We have examined platelet coagulant activities, volumes, and aggregate ratios in 23 patients with chest pain, seven of whom had normal coronary angiograms (group I) and 16 of whom had angiographically proven coronary artery disease (Group II). There were no significant differences in the mean values for platelet volume or platelet aggregate ratios between the two groups. The platelet coagulant activities concerned with initiation and the early stages of intrinsic coagulation were significantly increased in patients in group II as compared with those in group I. No significant differences were noted between the two groups with respect to prothrombin time, partial thromboplastin time, and plasma levels of fibrinogen and coagulation factors V and VIII. However, the mean activity in plasma of antithrombin III (but not the level of antithrombin III antigen) was significantly lower in patients of group II compared with group I. Overall, our observations provide evidence for an enhanced contribution to the intrinsic coagulation system in patients with coronary artery disease. The platelet coagulant hyperactivity noted in these patients may reflect a role of platelets in the pathogenesis of coronary artery disease or may be secondary to the underlying arterial disease.

Rao, A. K., *Walsh, P. N. et al.*

Circulation 69(1):15-21, 1984.

Other support: Ciba-Geigy Corporation and U. S. Department of Health and Human Services.

From the Thrombosis Research Center and the Cardiology Section, Department of Medicine, Temple University Health Sciences Center, Philadelphia.

ACTIVATION OF COAGULATION FACTORS ON THE SURFACE OF PLATELETS

Platelet physiology and coagulation biochemistry have traditionally been studied and conceptualized as separate and distinct entities. This brief summary of platelet-coagulant protein interactions presents an alternative viewpoint which emphasizes the effects of platelets in coagulation and the effects of coagulation proteins on platelet physiology. These interrelationships are so intimate and important that any consideration of the hemostasis mechanism that fails to take them into account is meaningless.

Walsh, P. N.

Haematologia 17(1):55-66, 1984.

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Other support: Public Health Service.

From the Thrombosis Research Center, Department of Medicine, Temple University School of Medicine, Philadelphia.

PLATELET COAGULANT ACTIVITIES IN DIABETES MELLITUS: EVIDENCE FOR RELATIONSHIP BETWEEN PLATELET COAGULANT HYPERACTIVITY AND PLATELET VOLUME

Platelets have been shown to be capable of initiating and promoting some of the reactions in intrinsic coagulation and have been implicated in the pathogenesis of vascular lesions in diabetes mellitus. We have examined the contribution of platelets to the coagulation reactions in 39 patients having diabetes mellitus with and without retinopathy [background retinopathy (BR) or proliferative retinopathy (PR)]. 18 patients with nonvascular eye diseases (patient controls), and 38 normal subjects. In comparison with control patients, significant elevations were noted in four of the five assays for platelet coagulant activities in diabetic patients with PR and in three of five assays in patients with BR. Thus, platelet coagulant hyperactivity is present in diabetics with retinopathy. Significant elevations were noted in two of the assays in patients without retinopathy (NR), suggesting that platelet coagulant hyperactivity may not necessarily be secondary to the vasculopathy. The predominant abnormalities noted were in assays reflecting the contribution of platelets to the early stages of intrinsic coagulation rather than to later stages of prothrombin activation. In addition, the mean platelet volume was found to be significantly greater in patients with PR and BR but not NR than in normal controls. A positive linear correlation ($r = 0.81$) was noted between the mean of the coagulant activities and platelet volume in diabetic subjects, suggesting a hitherto undescribed relationship between platelet coagulant activities and platelet volume.

Rao, A. K., Goldberg, R. E. and *Walsh, P. N.*

The Journal of Laboratory and Clinical Medicine 103(1):82-92, 1984.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Department of Medicine, Temple University School of Medicine, and Retinal Vascular Unit, Wille Eye Hospital, Philadelphia.

THE POSSIBLE ROLE OF PLATELETS IN BYPASSING THE CONTACT PHASE OF BLOOD COAGULATION

Data presented herein and previously support an active role for platelets in promoting the interaction and activation of the coagulation proteins of the contact phase of intrinsic coagulation. The platelet membrane, activated by ADP collagen or thrombin, can promote the proteolytic activation of factor XII to factor XIIa in the presence of kallikrein and high molecular weight kininogen. The zymogen factor XI associates with high molecular weigh kininogen in plasma and becomes bound to a site on the membrane of thrombin or collagen activated platelets. Thereafter, platelet bound factor XI can be proteolytically activated to factor XIa either in the presence of factor XIIa or in the presence of kallikrein. These observations could explain the absence of bleeding complications in patients with factor XII deficiency. In addition, platelets contain a molecule which has a higher molecular weight than plasma factor XI and possibly consists

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of a tetramer of four identical subunits of 52,000 daltons each of which is functionally and immunologically similar to plasma factor XI. Since this molecule is present in the platelets of patients with severe plasma factor XI deficiency and no evidence of bleeding, we postulate that platelet factor XI can substitute for plasma factor XI in hemostasis and possibly account for the considerable variability in clinical severity observed in patients with factor XI deficiency.

Walsh, P. N., Tuszynski, G. P., Greengard, J. S., and Griffin, J. H.

Haematologia 17(2):169-178, 1984.

Other support: U. S. Department of Health, Education and Welfare.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia, and Scripps Research Institute, La Jolla, CA.

ASSOCIATION OF FIBRIN WITH THE PLATELET CYTOSKELETON

The authors have previously postulated that surface membrane proteins become specifically associated with the internal platelet cytoskeleton upon platelet activation. Four lines of evidence support this general hypothesis since we now show that platelet surface receptors for fibrin become specifically associated with the platelet Triton-insoluble cytoskeletons. 1) Fibrin was detected immunologically in the washed Triton-insoluble cytoskeletons of thrombin-activated platelets under conditions where fibrin polymerization and resultant precipitation were blocked with Gly-Pro-Arg-Pro, a synthetic peptide that inhibits polymerization of fibrin monomer. 2) Radiolabeled fibrin bound to thrombin-activated platelets and became associated with the cytoskeleton. 3) The amount of radiolabeled fibrin bound to thrombin-activated thrombasthenic platelets and their cytoskeletons amounted to about 20% of the fibrin bound to thrombin-activated control platelets and their cytoskeletons. 4) The association of fibrin with cytoskeletons and with the platelet surface was nearly quantitatively blocked by an antibody prepared against cytoskeletons (anti-C), an antibody against isolated membranes of Pronase-treated platelets (anti-M1), and a monoclonal antibody to the platelet surface glycoprotein complex, $GPII_b$-GPIII (anti-GPIII). These antibodies blocked ADP and thrombin-induced platelet aggregation as well as thrombin-induced clot retraction. Analysis of the immunoprecipitates obtained with anti-C, Anti-M1, and anti-GPIII from detergent extract of ^{125}I-surface labeled platelets revealed that these antibodies recognized $GPII_b$-GPIII. These data suggest that thrombin activation of platelets results in the specific association of fibrin with the platelet cytoskeleton, that this association may be mediated by the $GPII_b$-GPIII complex, and that these mechanisms may play an important role in platelet aggregation and clot retraction induced by thrombin.

Tuszynski, G. P., Kornecki, E., Cierniewski, C., Knight, L. C., Koshy, A., Srivastava, S., Niewiarowski, S., and *Walsh, P. N.*

The Journal of Biological Chemistry 259(8):5247-5254, 1984.

Other support: U. S. Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

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AMIDOLYTIC ASSAY OF HUMAN FACTOR XI IN PLASMA: COMPARISON WITH A COAGULANT ASSAY AND A NEW RAPID RADIOIMMUNOASSAY

The traditional coagulant assay for plasma factor XI suffers from a relatively high coefficient of variation, the need for rare congenitally deficient plasma, and a poor correlation between precision and sensitivity. We have developed a simple functional amidolytic assay for factor XI in plasma using the chromogenic substrate PyrGlu-Pro-Arg-*p*-nitroanalide (S-2366). After inactivation of α_1-antitrypsin, C1 inhibitor, and other plasma protease inhibitors with $CHCl_3$, plasma was incubated with kaolin, in the absence of added calcium, which limited the enzymes formed to those dependent on contact activation. Soybean trypsin inhibitor was used to mimize the action of kallikrein on the substrate. Once the reaction was complete, corn trypsin inhibitor was used to inactive factor XIIa, the enzyme generated by exposure of plasma to negatively charged surfaces which had activated the factor XI. The assay is highly specific for factor XI, since plasma total deficient in that zymogen yielded only 1%-3% of the enzymatic activity in normal plasma under identical conditions. The requirements for complete conversion of factor XI to XIa in plasma within 60 min were, respectively, factor XII, 0.6 U/ml. Prekallikrein was not an absolute requirement for complete activation but did accelerate the reaction. The intraassay coefficient of variation was 3.4%, and the mean of 35 normal plasmas was 1.00 U ± 0.24 SD. In addition, a new rapid radioimmunoassay was devised using staphylococcal protein A as the precipitating agent for a complex of factor XI antigen with monospecific rabbit antibody. The mean was 1.01 U ± 0.30 SD. The correlation coefficients for amidolytic *versus* coagulant and amidolytic *versus* radioimmunoassay were r = 0.95 for the former and 0.96 for the latter. Thus, a simple, accurate amidolytic assay and a radioimmunoassay have been devised for measuring factor XI in plasma that correlate well with the coagulant activity of factor XI, as determined in our laboratory.

Scott, C. F., Sinha, D., Seaman, F. S., *Walsh, P. N.*, and Colman, R. W.

Blood 63(1):42-50, 1984.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Hematology-Oncology Section, Department of Medicine, Temple University School of Medicine, Philadelphia.

INTERACTION OF COAGULATION PROTEINS WITH PLATELETS: POSSIBLE SIGNIFICANCE FOR MAINTENANCE OF HEMOSTASIS AND PATHOGENESIS OF THROMBOEMBOLISM

The purpose of this chapter is to present some of the evidence that platelets possess binding sites for a variety of coagulation proteins and that these specific platelet coagulant protein interactions can promote zymogen activations at various stages of intrinsic coagulations including the initiation of intrinsic coagulation. In addition, studies are presented that are consistent with the hypothesis that these interactions of coagulation proteins with platelets are important physiologically in promoting normal hemostasis and pathologically in predisposing to certain venous and arterial thrombotic diseases.

Walsh, P. N.

In: *Atherosclerosis Reviews* 12, Raven Press, NY, 1984, pp 129-143.

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Source: https://www.industrydocuments.ucsf.edu/docs/qqxk0000